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A CYTOGENETIC STUDY OF OFFTYPES IN A WINTER WHEAT, DAWSON'S GOLDEN CHAFF, INCLUDING A WHITE CHAFF MUTANT[1]

BY R. MERTON LOVE[2]

Abstract

A cytologically aberrant mutant, found in Dawson's Golden Chaff winter wheat, had white glumes that were significantly longer than normal and the plant was significantly shorter than normal. It was characterized by a telokinetic pair of chromosomes. Evidence from two other lines has shown that such chromosomes arise from fragmentation of either primary or secondary univalents.

The results, together with those obtained from speltoid and fatuoid studies, indicate that domestic species and varieties of cereals differ from their prototypes, not necessarily through gene substitution, but through the addition of genes that mask the effect of primitive genes still a part of the complex or through gene substitution in a chromosome other than that carrying the primitive gene in question.

Introduction

Lancaster wheat is bearded, has white chaff and red grains (8) and presumably was an introduction from the Mediterranean (3). Carleton (2) reported: "In 1862, in Mifflin County, Pa., Abraham Fultz, while passing through a field of Lancaster wheat, which is an awned variety, found three spikes of awnless wheat. He sowed the seed from these spikes the same year and continued sowing a larger amount each year until he had obtained sufficient seed to distribute it pretty well over the country." According to Carleton (1), Garrett Clawson of Seneca County, New York State, selected superior heads from a field of Fultz in 1865. Both a white- and a red-grained sort resulted and Clawson increased the white-grained variety. It became known as Seneca or Clawson wheat. In 1881, Robert Dawson of Paris, Ont., found one plant in a field of Seneca wheat that was quite distinct and much superior to the remainder of the crop (10). This was increased and given the name Dawson's Golden Chaff.[3]

The history of Dawson's Golden Chaff is interesting in view of the kinds of offtypes that arise spontaneously from time to time in seed stocks of this

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[3] *Called Dawson's in the United States.*

variety. Typically, Dawson's Golden Chaff is a soft, white-grained, awn-letted winter wheat with reddish-brown or golden glumes. Its resistance to Hessian fly gives it much importance in many wheat improvement projects. Offtypes include awned, red-grained, or white chaff variants, as well as dwarf types that are sterile.

In Canada, the registration of cereal varieties allows only one distinctly offtype plant in 10,000, and since plants with white chaff appear with a frequency that jeopardizes the maintenance of elite seed stocks, a cytogenetic study of the variety, and particularly of this mutant, was initiated. An abstract has been published (5) and this paper presents details and additional information that has been obtained.

Material and Methods

Thirty plants, chosen at random in a block of elite foundation material, were examined cytologically in 1935. At maturity all appeared to be normal (cf. Fig. 1), but two of them, *DGC* 3 and *DGC* 16, were characterized by irregularities during meiosis. That same year one white chaff offtype (*DGC* 31) occurred in the field and the progeny were grown and studied. The head is illustrated in Fig. 2.

All plants were spaced in the field or grown in pots in the greenhouse. Winter killing was severe the second and fourth years and resulted in very small field progenies. Meiotic material was collected, stored, and smeared as outlined previously (6). Photomicrographs were taken at magnifications of 520 and 1100×.

Results

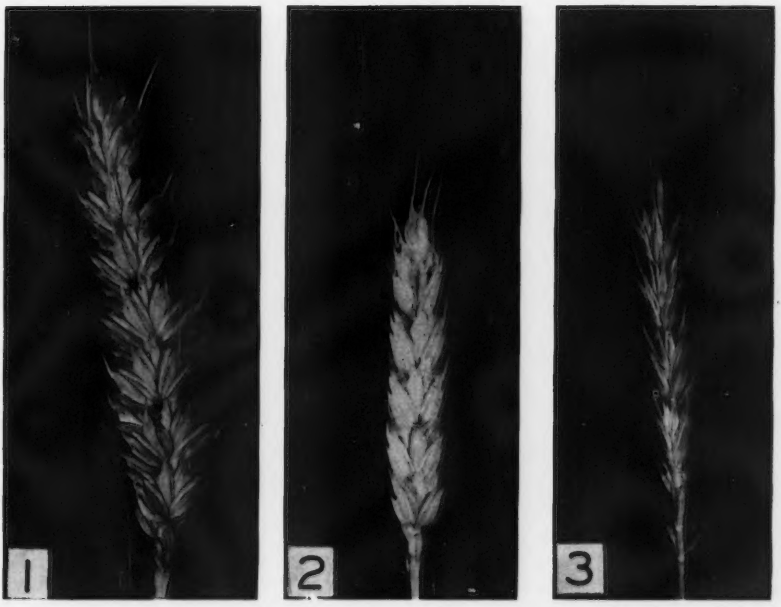
White Chaff Offtype, Line DGC 31

The white chaff plant found in the isolation field bred true for white chaff, except for three golden chaff plants to be discussed later. Twenty morphological characters were measured and it was found that white chaff plants had longer glumes (Table I) and were shorter plants (Table II) than normal. Although the percentage fruitfulness was not measured, the white chaff plants appeared to be less fertile than the normal plants.

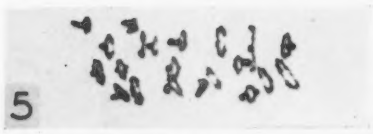
All the white chaff plants derived from *DGC* 31 that were examined cytologically had a telokinetic pair of chromosomes (Fig. 4). Members of the telokinetic bivalent failed to synapse in five of the 275 first metaphase plates examined. Less than three per cent of the pollen mother cells had any unpaired chromosomes at that stage. In normal Dawson's Golden Chaff all chromosomes have median or submedian kinetochores and about two per cent of the pollen mother cells display irregularities at first metaphase.

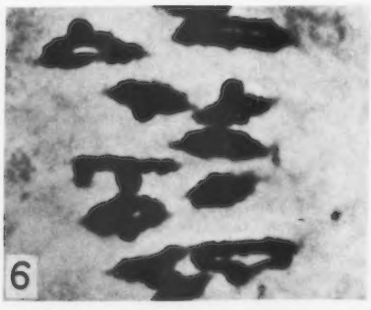
The small greenhouse progenies of the three golden chaff plants referred to above yielded 20 golden and five white chaff plants. Eleven of the plants that later proved to have golden chaff were examined cytologically. One was normal (Fig. 5) and 10 had a heteromorphic bivalent, one member of which was telokinetic (Figs. 6 and 7). The two white chaff plants examined had a

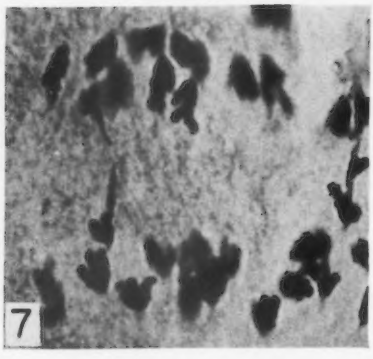
PLATE I

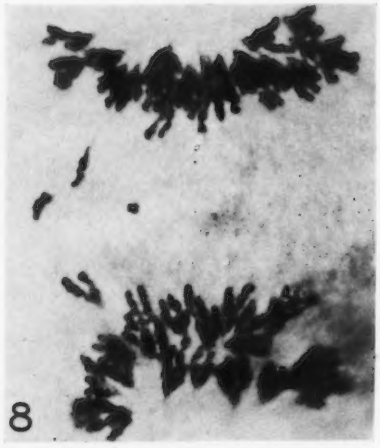


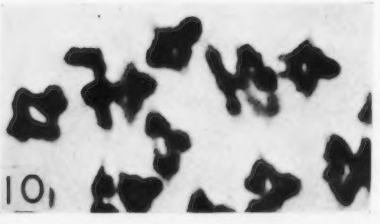
Photographs of wheat spikes. ×*1 approx.* FIG. 1. *Normal Dawson's Golden Chaff.* FIG. 2. *Cytologically deficient white chaff offtype.* FIG. 3. *Sterile dwarf offtype.*











Photomicrographs of pollen mother cells. Aceto-carmine smear preparations. FIGS. 5, 9, 11, *×520; others, ×1100.* FIG. 4. *Part of a pollen mother cell from a white chaff offtype, illustrating the chromosome pair homozygous for the loss of one arm (at left).* FIG. 5. *Normal 21 bivalents in Dawson's Golden Chaff.* FIGS. 6 AND 7. *Note the heteromorphic bivalent. This plant is phenotypically normal, but produces true-breeding normals, segregating normals, and white chaff plants.* FIG. 8. *Misdivision of a primary univalent in a 41-chromosome plant. This illustrates one way in which a telokinetic chromosome may arise. The plant is heterozygous for an inversion.* FIG. 9. *Primary and secondary (above the first metaphase plate) monosomes. The arms of the secondary monosome synapsed in about two-thirds of the cells in this plant.* FIG. 10. *The plant with this heteromorphic bivalent arose under observation, DGC Line 16.* FIG. 11. *First metaphase in an F_1 plant between homozygous deficient white chaff (see Fig. 4) and heterozygous deficient plant in DGC 16 (see Fig. 10). Note the two heteromorphic bivalents.*

TABLE I

COMPARISON OF WHITE CHAFF AND NORMAL GLUME LENGTHS, LINE *DGC* 31

Family	Number of plants		Glume length			
			White chaff		Golden chaff	
	White chaff	Golden chaff	Range, mm.	Mean, mm.	Range, mm.	Mean, mm.
Greenhouse						
117	2	7	–	9.0	7.6 – 8.3	8.1
118	1	7	–	8.5	7.5 – 8.2	8.1
119	2	6	8.8 – 9.0	8.9	7.8 – 8.0	7.9
Field*						
54	1	18	–	8.3	6.6 – 8.0	7.4
55	1	18	–	8.2	7.1 – 7.8	7.5
58	2	16	8.0 – 8.3	8.2	7.0 – 8.1	7.6
59	2	8	–	8.1	7.2 – 8.0	7.5
61	1	13	–	8.3	7.1 – 8.0	7.6
64	1	14	–	8.3	7.3 – 7.9	7.5

** Only plants from which meiotic material was not collected were used in the glume length and plant height studies of field progenies.*

TABLE II

COMPARISON OF HEIGHTS OF WHITE CHAFF AND NORMAL SEGREGATES, LINE *DGC* 31

Family	Height			
	Plants with white chaff		Plants with golden chaff	
	Range, in.	Mean, in.	Range, in.	Mean, in.
Greenhouse				
117	30 – 31	30.5	34 – 44	40.2
118	–	30	32 – 43	37.6
119	27 – 39	33.0	29 – 41	35.2
Field*				
54	–	32	34 – 47	38.1
55	–	35	38 – 49	44.6
58	–	34.0	37 – 51	41.1
59	30 – 31	30.5	35 – 44	40.1
61	–	38	32 – 43	40.0
64	–	31	33 – 42	39.0

** See footnote to Table I.*

telokinetic pair of chromosomes similar to that observed in the progenies of the original white chaff plant. It seems obvious that the three golden chaff plants found in the progeny row of *DGC* 31 resulted from natural crossing with normal plants. Phenotypically, heterozygotes cannot be distinguished from normals.

The following year six progenies from heterozygotes were grown, comprising a total of 158 plants, 145 of which were phenotypically normal and 13 had white chaff. Twenty-five plants were examined cytologically, 23 with golden and two with white chaff. Seven of the golden chaff plants were normal and 16 had the heteromorphic bivalent. Both white chaff plants had the telokinetic bivalent.

Progenies of the two white chaff plants bred true, as did those of the two golden chaff plants carried on.

For the glume length studies, the first empty glume of the seventh spikelet from the bottom of the spike was measured in millimetres. In nine families, 120 individuals were measured. Table I gives the data by families. In every family (except No. 58) all the white chaff plants had longer glumes than their golden chaff siblings. When the white chaff glume length is paired with the mean of sister golden chaff glume lengths, the Z value obtained is 3.8421, which, according to Student's table for nine pairs of values (4) gives odds exceeding 10,000 : 1 against such differences in length being due to chance.

Treating the heights of the above plants the same way (Table II) the Z value is 2.4571, which also gives odds exceeding 10,000 : 1 against such differences in height being due to chance, although there was overlapping in Families 119 and 61.

It is quite clear from the results obtained from white chaff offtype Line 31 that the white chaff character is due to the absence of one arm of a chromosome, but it provides no clue concerning the origin of such a deficiency. The study of lines *DGC* 3 and 16 provided such evidence.

Cytologically Aberrant Line DGC 3

Of a total of 39 pollen mother cells examined at first metaphase in Plant *DGC* 3, 18 were irregular with 20 bivalents and two univalents present. They appeared to be the same length, although one cannot be certain of slight differences in lengths of wheat chromosomes at meiosis. However, this amount of meiotic irregularity compares unfavourably with that found in the majority of the 30 plants examined that year, which had on the average less than two irregular pollen mother cells in a hundred. *DGC* 3 was normal phenotypically, and so were all 24 plants derived from it, but six of the seven plants examined cytologically were monosomic with $2n = 41$.

Owing to the severe winter killing, no field studies of the second generation could be made. The following data were obtained from small greenhouse progenies. Three plants, the progeny of the 42-chromosome D_1, had 21 bivalents and were normal phenotypically. They were fertile and were 37, 38, and 40 in. tall.

A total of 35 plants were grown from the six monosomics: 28 appeared normal and seven were dwarf, sterile plants. The height of the dwarfs varied from 20 to 30 in. with a mean of 25.3 in. One of the dwarfs set one seed that developed into a tall, fertile, apparently normal plant. Although it was

not examined cytologically, it is likely that it was the result of a natural cross with a 42-chromosome plant and had 41 chromosomes. A typical dwarf spike is illustrated in Fig. 3. Three of the dwarfs were examined cytologically. Two had $2n = 40$, forming 19 bivalents and two univalents in approximately one-third of the pollen mother cells. As many as four and six univalents were noted occasionally. In the remainder of the cells 20 bivalents were seen. The third dwarf, also sterile, had 20 bivalents and one secondary monosome. In this plant members of bivalents rarely remained unpaired and the two arms of the univalent were synapsed in 23 of the 25 cells examined.

Nine of the 28 apparently normal plants were examined cytologically. Two had $2n = 42$, were fertile, and were 34 and 37 in. tall. The remaining seven plants were monosomic and as well as could be ascertained from meiotic preparations, the same chromosome was monosomic in all. Pairing at first metaphase was quite regular, 20 bivalents and one univalent occurring in 95% of the 175 pollen mother cells examined. More than three unpaired chromosomes were not seen. Fragmentation of the univalent at the kinetochore was noted in two of the few cells examined at first anaphase (Fig. 8). The heights of the seven plants varied from 30 to 48 in., with a mean of 37 in. They were fertile.

Cytologically Aberrant Line DGC 16

Plant *DGC* 16, which was normal phenotypically, had 42 chromosomes, but in 57 of the 60 pollen mother cells examined at least two univalents were present. In three cells 21 bivalents were counted.

Ten of the 12 progeny of *DGC* 16 grown in the greenhouse were fertile and normal phenotypically, varying in height from 31 to 42 in., with a mean of 36.9 in. The remaining two plants were sterile dwarfs, 23 and 24 in. tall. Three of the four "normals" examined cytologically had 41 chromosomes. Siblings 2 and 4 were typical 41-chromosome plants, 31 and 42 in. tall, respectively. The univalent in sibling 3 (31 in. tall) formed a ring at first metaphase in about half the cells and pairing of the remaining 40 chromosomes was normal.

Six progeny were grown from sibling 2 and two of them were examined cytologically. One of these was 23 in. tall, partially fertile, and had 42 chromosomes including a primary and a secondary monosome (Fig. 9). Pairing behaviour in this plant was as follows:

21 pairs—11 cells;
20 pairs and 2 univalents—22 cells;
19 pairs and 4 univalents—3 cells.

Where the two unequal chromosomes paired, synapsis was end-to-end, or, in two instances, the arms of the secondary synapsed and the primary paired terminally with them. Both first and second metaphases showed much fragmentation of the univalents. The second plant was a typical 41-chromosome plant, 31 in. tall and fertile. Of the four plants not examined cyto-

logically, three appeared normal and were 31 to 38 in. tall. The fourth was a dwarf, sterile plant, 21 in. in height.

Of the five plants grown from sibling 3, the one examined cytologically had 42 chromosomes including a heteromorphic pair, one member of which possessed a terminal kinetochore (Fig. 10). This, and two sister plants, were normal phenotypically, but two siblings were sterile dwarfs.

From the plant with the heteromorphic bivalent discussed in the preceding paragraph a total of 56 progeny were grown, 54 of which were normal phenotypically and two were sterile dwarfs. Of the 37 that were examined cytologically, 25 were normal with 21 pairs, 11 were characterized by the heteromorphic bivalent, and one was homozygous for the deficiency. The first two cytological types could not be distinguished phenotypically; the one with the homozygous deficiency was a sterile dwarf.

Attempts were made to hybridize the homozygous deficient white chaff type with the above discussed deficient type. Heterozygotes had to be used as the homozygous deficient plants in Line 16 were very weak. One hybrid resulted that had two heteromorphic chromosomes (Fig. 11). It appeared to be normal and had golden chaff. This hybrid provided definite evidence that the telokinetic chromosome in white chaff line *DGC* 31 was not the same as that in Line 16.

Discussion

Up to the present time, most cytogenetic studies on mutants in wheat have been concerned with speltoid and related types. The fatuoid problem in oats is an analogous situation. In both these types it has been shown by Huskins and co-workers (results to be published shortly) that the occurrence of the speltoid and fatuoid phenotypes is the result of the loss of particular segments of a certain chromosome, and as a consequence of the loss, the genes for the speltoid and fatuoid complexes (genes that are still present in the cultivated varieties) are allowed expression.

The results reported herein on chaff colour in a winter wheat variety have the same explanation. It has been seen from the history of Dawson's Golden Chaff that the earliest progenitor on record, Lancaster wheat, had white glumes. The gene or genes for white chaff are still present in the variety but they are masked by the presence of a gene or genes on one arm of the chromosome under consideration. This arm also bears genes affecting glume length and plant height.

Of more practical (and theoretical) interest than the actual offtype itself is the manner of its origin. There can be little doubt that, at least in this variety, the offtype is referable to meiotic instability. Studies on this and many other wheat varieties by the writer have shown that the presence of unpaired chromosomes in, say, 10% of the pollen mother cells of a plant invariably leads to a relatively high proportion of 41-chromosome individuals in the next generation (6, and unpublished data of the writer). From the

practical standpoint it is important to note that the monosomics can rarely, if ever, be eliminated by "roguing" for they are phenotypically normal.

Furthermore, the misdivision of univalents, resulting in fragmentation at or in the kinetochore region, is common at the first meiotic anaphase. This is true of univalents whether they be primary, secondary, or telokinetic. The same conditions obtain with members of a pair that frequently fail to synapse. In the material under investigation, the first aberrant chromosomal type to arise was a univalent that formed a ring at first metaphase; it was presumably an isochromosome. Twenty-six plants that were derived from primary monosomics or from 42-chromosome plants with a high frequency of unpaired chromosomes were examined in this study: three of the 26 had a secondary and none had a telokinetic chromosome. This does not mean, however, that telokinetic chromosomes cannot arise directly from primary univalents in wheat for such have been shown to occur (6) and Fig. 8 illustrates clearly how a telokinetic chromosome may originate from a primary monosome. Additional illustrations of the misdivision of primary univalents (in F_1 pentaploid wheat hybrids) have been published previously (7).

One plant that was derived from a 41-chromosome plant with a secondary univalent had a telokinetic chromosome, indicating that the secondaries, too, are unstable in wheat. This study corroborated by previous work (see Love (6) for illustrations) has shown clearly that where a secondary is present as a univalent misdivision frequently occurs.

Thirty-seven plants derived from a plant with a telokinetic chromosome (that formed a heteromorphic bivalent with its normal homologue) in Line 16 and 38 such plants in Line 31 were examined cytologically and no secondaries were found. The cytological types obtained totalled 25 normal, 11 with a heteromorphic bivalent, and one with a telokinetic bivalent in Line 16, and eight normal, 26 heteromorphic, and four telokinetic in Line 31, so there is obviously some elimination of the telokinetic chromosome, and especially in Line 16. It is the opinion of many workers (reviewed by Rhoades (9)) that a terminal kinetochore is unstable and that this instability probably accounts "for the fact that telocentric chromosomes are rarely, if ever, found in the normal chromosome complement of any organism" (9). In view of the above evidence, it might seem that the only conclusion to be drawn regarding the deficient chromosome in the white chaff offtype is that it does not possess a terminal kinetochore. The chromosome has not been studied in prophases but the manner of origin of similar chromosomes in wheat and the appearance of such chromosomes at first and second anaphases suggests that they are truly telokinetic.

Acknowledgments

The writer wishes to thank Dr. L. H. Newman, Dominion Cerealist, and Mr. A. G. O. Whiteside, in charge of Winter Wheat Investigations, for suggesting this study.

References

1. CARLETON, M. A. U.S. Dept. Agr., Div. Veg. Physiol. Path. Bull. 24. 1900.
2. CARLETON, M. A. The small grains. Macmillan Company, New York. 1916.
3. CLARK, J. A. and BAYLES, B. B. U.S. Dept. Agr. Tech. Bull. 459. 1935.
4. HAYES, H. K. and GARBER, R. J. Breeding crop plants. 2nd ed. McGraw-Hill Book Company, Inc., New York. 1927.
5. LOVE, R. M. Abstract. Genetics, 23 : 157. 1938.
6. LOVE, R. M. Can. J. Research, C, 18 : 415-434. 1940.
7. LOVE, R. M. Can. J. Research, C, 19 : 351-369. 1941.
8. PERCIVAL, J. The wheat plant. Duckworth and Company, London. 1921.
9. RHOADES, M. M. Genetics, 25 : 483-520. 1940.
10. SHAW, T. and ZAVITZ, C. A. Ontario Agr. Coll. Expt. Sta. Bull. 90. 1893.

MICRO TESTS OF ALIMENTARY PASTES

III. THE DIFFERENTIAL RESPONSE OF VARIETIES TO PROCESSING METHODS[1]

By J. Ansel Anderson[2] and R. L. Cunningham[3]

Abstract

The differential response of durum wheat varieties to processing methods, used in the micro test, was studied in a factorial investigation involving four varieties and 27 methods representing the factorial combinations of three absorption levels, three mixing times, and three pressures. Changes in the quality of the micro disks of alimentary paste were measured in terms of the optical absorption coefficient (opacity), colour characteristics (black, yellow, red, and white), and pigment content. Significant interactions between varieties and the three processing factors were demonstrated; different processing methods placed the semolinas in different orders with respect to most of the quality characteristics. It is concluded that it will be difficult to devise a standard micro test for alimentary pastes that will be widely accepted. However, larger interactions with methods will not occur with all pairs of varieties, and it will generally be possible to demonstrate consistent differences between most varieties with respect to at least one or two quality characteristics.

It has been shown by Cunningham and Anderson (2) that, in the micro test for alimentary pastes developed by Fifield, Smith, and Hayes (3) and modified by Cunningham and Anderson (1), wide differences in paste properties can be obtained by modifying the processing conditions. The development of a standard procedure for testing the quality of new varieties of durum wheat therefore presents certain difficulties; for it is improbable that varieties will be placed in exactly the same rank order by different processing methods; and any standard method that is adopted will therefore favour certain varieties and penalize others. Similar problems have been met in work on baking and malting tests, and in many other fields. In a sense these problems are insoluble; cereal chemists cannot agree on standard methods, and each man selects the modification that best suits his own purposes. But there is little confusion; for with the baking test, and to a lesser extent with the malting test, there is a large background of information, and the chemist who knows his tools is unlikely to misuse them or misinterpret his results. Tests for alimentary pastes have been investigated much less extensively, and more information is required before their utility can be considered as firmly established. The study reported in this paper is designed to throw further light on the difficulties involved in developing and using a micro test for assessing the quality of durum wheats for making alimentary pastes.

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In a sense, this paper is an interim report dealing principally with the interaction between varieties and processing methods. The study is part of a continuing series; it looks to the selection of three or four processing methods with which varieties react differently. These will be used in further investigations in which it is our intention to compare the interaction between varieties and methods with the interaction between varieties and environments. It is apparent that this must be the ultimate objective; for if the differential effect of methods on varieties is small by comparison with the differential effect of environment on varieties, then it will be possible to neglect the former—which can be classed as the experimental error of the method—in assessing the average performance of varieties over any area, or period of time, in which wide variations in environmental conditions occur.

Materials and Methods

The four semolinas used in the investigation were milled from four samples of durum wheat representing three new varieties under study in Canada in 1941 and a composite sample of the 1940 crop of Western Canadian amber durum wheat. The varieties were (*A*) *R.L.* 1491, a variety from North Dakota (*L.D.* 34) representing a backcross of Mindum to a cross between Mindum and Pentad; (*B*) *R.L.* 1612, a cross between Iumillo and Mindum; and (*C*) *R.L.* 1183.7, a single plant selection of another derivative from a cross between Iumillo and Mindum. Semolina (*D*) was milled from a composite of the 1940 crop grading 2 *C.W.* amber durum; it consisted principally of Mindum wheat; and, for purposes of simplifying nomenclature, it is referred to as variety *D* throughout the remainder of this paper. The history of the samples is of no particular importance; they were selected because the differences in quality among them covered a suitable range for the investigation of possible interactions between varieties and methods.

The methods and equipment used for making micro disks of alimentary pastes in this laboratory have been described by Cunningham and Anderson (1). This paper also contains descriptions of methods for assessing the quality characteristics of the disks. These are measurement of the optical absorption coefficient (or opacity), the colour characteristics, and the pigment content. It should be noted that the colour characteristics are reported by a new method (1): black is recorded in the usual manner as the percentage used in the matching disk; but yellow, red, and white are reported as percentages of the non-black component of the matching disk, rather than as percentages of the whole disk.

Twenty-seven processing methods were used in the investigation. These represented the factorial combinations of three absorption levels (28, 30, and 32%), three mixing times (40, 72, and 100 sec.), and three pressures (700, 1000, and 1500 lb./sq. in. on the gauge of the Carver press).

Average Differences Between Varieties

Before considering the principal feature of the investigations, the interactions between varieties and methods, it is advisable to consider the average quality characteristics of the four varieties. It is apparent that if the four varieties had been identical in average quality there would have been little opportunity to illustrate interactions with processing factors. The samples were selected with this point in mind and cover a moderately wide range of quality characteristics.

The data shown in Table I represent the means over all 27 processing methods for disks made from each variety. Opacities (optical absorption coefficients, *K*) are shown in the first column of data. Variety *A* was quite opaque, *B* and *C* gave intermediate values, and *D* was moderately translucent. There are also appreciable differences with respect to colour characteristics: *A* and *D* show high values for black; *C* and *D* show high values for yellow; *B* gives a much lower red value than the other three samples; and *A* and *B* show considerably higher values for white than *C* and *D*. With respect to pigment content, *C* is much higher than *D*, and *A* gives the lowest value.

TABLE I

QUALITY CHARACTERISTICS OF MICRO DISKS MADE FROM EACH VARIETY. MEANS OVER ALL PROCEDURES

Semolina	Opacity coeff., *K*	Black segment, %	Non-black component, %			Pigment content, p.p.m.
			Yellow	Red	White	
(*A*) *R.L.* 1491	4.98	37.9	42.3	17.1	40.6	2.43
(*B*) *R.L.* 1612	4.79	32.3	46.3	14.4	39.3	2.65
(*C*) *R.L.* 1183.7	4.73	35.4	50.1	18.2	31.7	3.93
(*D*) Crop composite	4.25	38.4	49.9	17.5	32.6	2.74

It is thought that the opacity should be as low as possible; for the more translucent the paste the clearer and deeper its colour appears. The interpretation of the percentage of black required in the matching disk is not simple. On the one hand, more black may be required merely because the disk is dull or grayish; and, on the other hand, the more translucent the paste, the less light will be reflected from it and the higher the amount of black required in matching it. Whereas dullness and grayness are bad qualities, translucency is highly desirable; but these two qualities have essentially the same effect on the black value, which complicates its interpretation. It is thought that the percentage of yellow should be as high as possible and that the percentage of red should also be reasonably high. It appears that the combination of a high yellow value with some red produces the typical amber yellow colour that is considered so desirable in macaroni. In the estimation of the authors, normal variations found in the value for white, as they calculate it, have little effect on colour quality, and can be disregarded. With respect to pigment content, it seems clear that this value should be as high as possible.

When interpreted in these terms, the data in Table I illustrate some interesting points. It appears that the black value was high for Variety *A* largely because the disk was dull, whereas the black value was high for Variety *D* principally because the disk was less opaque. It will be observed that *C* and *D* gave essentially the same values for percentage yellow in spite of the fact that the pigment content of *C* is a great deal higher than that of *D*. One of the explanations is that the average disk for *D* was appreciably less opaque than the average disk for *C*, and this caused the former to appear yellower. On the other hand, it should be noted that, if yellow values had been reported by the standard procedure rather than as percentages of the non-black component, the spread between the yellow values for *C* and *D* would have been greater.

It is difficult to rate the four samples with respect to quality as a whole. Because of its high opacity and low pigment content, Variety *A* was very low in percentage yellow, and it therefore appears to be the least satisfactory of the four varieties. Variety *B* is slightly superior to *A* in these three respects, and we should not be inclined to discount it heavily for its somewhat low red value. As between *C* and *D*, the latter is considered slightly superior, largely on account of its greater translucency. But the high pigment value of Variety *C* is very attractive, particularly from the point of view of a plant breeder selecting parent varieties for new crosses.

On the whole it appears that these four samples represent an average cross-section of the type of materials that plant breeders submit to the laboratory for quality tests. They therefore form a suitable series for the investigation of the interactions between varieties and processing methods.

Differential Effect of Methods on Semolinas

The results of analyses of variance can be conveniently used to select those interactions between semolinas and processing factors that are sufficiently large to merit more detailed study. Table II contains the pertinent statistics. These represent part of the analysis of variance for each of the quality charac-

TABLE II

ANALYSES OF VARIANCE

Variance due to:	Degrees of freedom	Mean squares					
		Opacity coeff., *K*	Black segment	Non-black component			Pigment content
				Yellow	Red	White	
Semolina	3	2.60	210.5*	362.9**	76.4*	556.1**	12.243**
Sem. × absorption	6	0.08	3.2	4.9**	1.0	8.0*	0.002
Sem. × mixing	6	0.19*	5.0	2.2	3.8**	4.9	0.033**
Sem. × pressure	6	1.15**	39.3**	7.2**	15.6**	37.1**	0.004
Error	68	0.07	4.0	1.5	1.2	3.0	0.002

Note: (*) and (**) denote that the 5% and 1% levels of significance were exceeded.

teristics of the disks, namely: the variance due to varieties; the variance due to the interaction between varieties and the principal processing factors; and the error variance, which represents the second and third order interactions between varieties and processing factors. The remaining portions of the analyses (i.e., the variance due to absorption, mixing time, pressure, and the interactions between these factors) are given in a later table.

The significance of the mean square for varieties is tested against the largest of the interactions between varieties and processing factors, which is that for varieties and pressure for all quality characteristics except pigment content. It will be observed that the mean square for varieties is significantly greater than the interaction for all characteristics except opacity. With this single exception the data therefore show that there were significant differences between the varieties; but it does not necessarily follow that all of the varieties differed with respect to each quality characteristic; and this point will require further study.

Turning to the interactions, it is found that 10 of these are significant, and that in seven instances the significance attains the 1% level. For all characteristics except pigment content, the interaction between varieties and pressures is highly significant and larger than the remaining two interactions. Pigment content presents an exception, as the interaction between varieties and pressure is not significant, whereas the interaction between varieties and mixing times is. The interaction between mixing time and varieties is also significant for opacity and for the red colour, and significant interactions between varieties and absorption are recorded for yellow and white.

These 10 significant interactions, together with their relation to the significance of the difference between varieties, can be studied conveniently by means of the histograms presented in Fig. 1. These are divided into three groups: the group dealing with pressure appears on the left; that for absorption is shown in the upper right-hand corner; and that for mixing time is shown in the lower right-hand corner. Under the heading of pressure, the first row of three histograms shows the interaction between pressure and varieties with respect to percentage yellow; the first histogram represents 700, the second 1000, and the third 1500 lb. per sq. in. For each of the three histograms, the four columns represent the four varieties *A*, *B*, *C*, and *D*, arranged in that order, as indicated by the letters at the bottom of the figure. Interactions with respect to percentage white, percentage black, percentage red, opacity, and pigment content, are shown by successive sets of histograms appearing in rows one below the other in the figure.

As the reader can study the individual sets of histograms for himself, it does not seem necessary to deal with each of them in detail. However, two or three typical sets may be examined with advantage, and short comments may be made on the remaining sets. The bottom three histograms on the left hand side, representing the differential effect of pressure on varieties with respect to opacity, are particularly interesting because they represent the only case in which the mean square due to varieties is not significantly greater

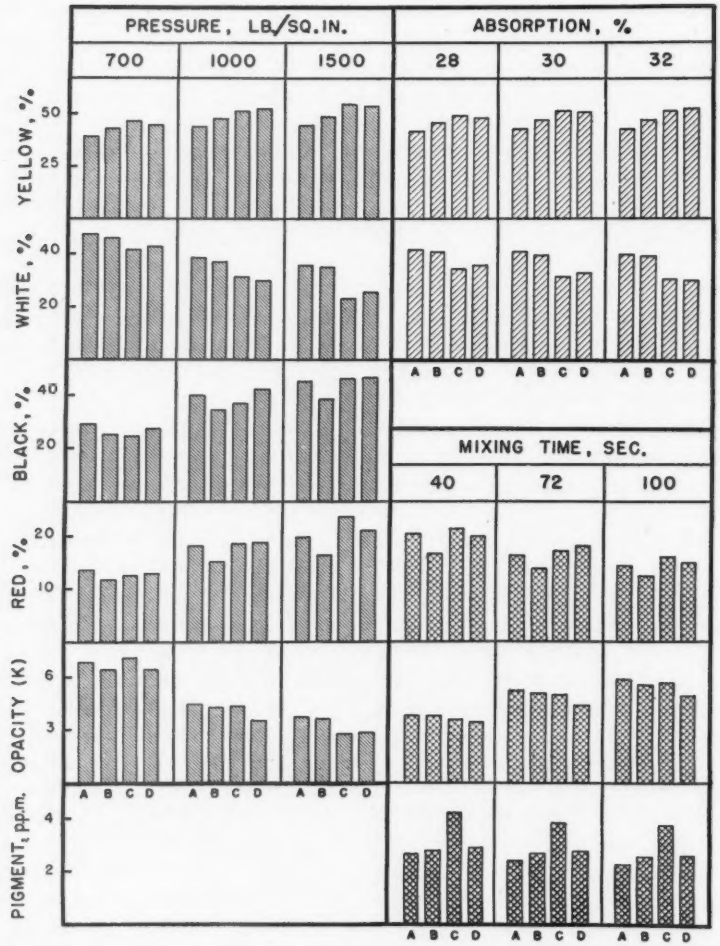

FIG. 1. *Histograms illustrating interactions between varieties (A, B, C, and D) and processing factors: pressure (left), absorption (top right), and mixing time (bottom right).*

than the interaction. The explanation is that the varieties fall in different orders with respect to opacity when processed at different pressures. At 700 lb. Variety *C* shows the highest opacity, whereas at 1000 lb. its value is intermediate, and at 1500 lb. it actually shows the lowest value. Variety *A* is consistently higher in opacity than *B*, but whereas the difference is appreciable at 700 lb., it is very small at the two highest pressures. The reverse situation occurs with respect to Varieties *B* and *D*; these have essentially the same value at 700 lb., but *B* is distinctly more opaque than *D* at both the higher pressures.

Consideration of the data as a whole suggests that even the most optimistic interpretation would result only in the conclusion that Variety *A* is probably inherently more opaque than Variety *D*, irrespective of the pressure used in processing. However, the analysis of variance suggests that this conclusion must be accepted with caution.

With respect to percentage red, the situation is somewhat similar; here again Variety *C* is responsible for the major portion of the interaction between pressures and varieties. It is also impossible to say that a difference exists between Varieties *A* and *D*; but it is clear that *B* is less red than the other three varieties at all three pressures. The analysis of variance shows that the mean square due to varieties attains the 5% level of significance. In passing, it may be noted that the interaction between mixing time and varieties with respect to percentage red (the three histograms to the right on the same line) is of essentially the same type as the interaction last discussed; it will be noted particularly that Variety *C* is again responsible for the major part of the interaction.

The histograms for percentage yellow, shown on the top row, illustrate an intermediate situation. Both sets, i.e., varieties × pressure and varieties × absorption, can be described together. Varieties *A*, *B*, and *D* hold their relative positions comparatively uniformly at all three pressures and again at all three absorptions. In addition, Variety *C* is consistently yellower than Varieties *A* and *B*; but its position changes with respect to *D*, so that it is impossible to say that any consistent difference in percentage yellow exists between Varieties *C* and *D*. Essentially the same description also fits the interactions with respect to percentage white: *A*, *B*, and *D* hold their relative positions, whereas the difference between *C* and *D* is not consistent, either at different pressures or at different absorptions.

The interaction between varieties and pressures with respect to percentage black is not vastly different from, though somewhat greater than, the interactions discussed in the preceding paragraph. At first sight, it appears that *A*, *B*, and *D* hold their relative positions at all pressures; but closer inspection shows that this statement is not true with respect to *A* and *D*, since the latter is less black than the former at 700 lb., whereas it is blacker at the remaining two pressures. Variety *C* is again responsible for most of the interaction; its position cannot be established with certainty with respect to any of the other three varieties.

The two sets of three histograms in the lower right hand corner of the figure remain to be discussed. Both relate to the interaction between mixing time and varieties, and in neither instance is the interaction very large. With respect to opacity, it is clear that Variety *A* is consistently more opaque than *D* at all three mixing times, and that Varieties *B* and *C* tend to have intermediate values. However, it will be noted that *B* is equal to *A* with 40 sec. mixing time, and that the relative positions of *B* and *C* change with increasing mixing time. In the last set of histograms, which relate to pigment content, all four varieties fall in the same order at each of the three mixing times. The

interaction is actually very small, arising from minute differences in the relative placings of varieties. Accordingly, the fact that it is significant is of little practical importance and indicates a low experimental error rather than a large interaction. In this respect it is pertinent to recall that the determinations of pigment content and of opacity are objective and precise, whereas the measurements of colours are subjective and therefore much less reproducible. In consequence, it is easier to demonstrate significant interactions with respect to pigment content and opacity.

As the number of samples used in the investigation was comparatively small, some caution must be exercised in drawing general conclusions with respect to the interactions between varieties and processing factors. Nevertheless, some interesting observations can be made. It is clear that Variety *C* is responsible for the major portion of most of the interactions. This seems peculiar in view of the fact that it is so closely related to Variety *B*; both represent crosses between Iumillo and Mindum. If the major portions of the interactions had been caused by Samples *A* or *D*, which represent a variety and a crop composite not closely related to each other or to *B* or *C*, the situation might have appeared simpler to explain. Under the existing circumstances it must be assumed that interactions can be expected, between varieties and processing methods, among any set of varieties, no matter how closely they are related. Nevertheless the data do not suggest that an insoluble problem exists. In all instances except one—the interaction between varieties and pressure with respect to opacity—it was possible to show that certain pairs of varieties differed consistently, irrespective of the processing methods adopted. In general, this is a situation that must be expected when new varieties and selections developed by plant breeders are tested in the laboratory. It may be concluded that it will always be necessary to examine the data in detail, and that it will not often be possible to make clear cut distinctions between each pair of varieties with respect to all quality characteristics. It is for this reason that in this laboratory we are now inclined to believe that the use of a total colour score, which tends to mask differences in individual colours by grouping them together, will not prove profitable in the study of wheats submitted for test by plant breeders. The major conclusion to be drawn from this part of the study is that, since different processing methods tend to place certain varieties in different order with respect to various quality characteristics, it will be difficult to establish a standard procedure for testing varieties that will be widely accepted.

Average Effect of Processing Factors

The effects on the finished micro disk of changing a number of processing factors were discussed at considerable length in the second paper of this series (2). However, in that paper changes in disk quality were discussed only in terms of changes in opacity. It was inferred that corresponding changes occur in the colour characteristics of the disks, but no data were

reported to substantiate this statement. It is therefore pertinent to report such data in the present paper. They are given in Table III.

TABLE III

MEAN EFFECTS OF PRINCIPAL PROCESSING FACTORS ON DISK PROPERTIES

Processing factor	Level	Opacity coeff., K	Black segment, %	Non-black component, %			Pigment content, p.p.m.
				Yellow	Red	White	
Absorption, %	28	5.14	34.5	46.0	16.3	37.7	2.94
	30	4.67	36.2	47.5	16.6	35.9	2.94
	32	4.26	37.4	48.0	17.5	34.5	2.93
Mixing time, sec.	40	3.66	40.5	50.1	19.5	30.4	3.13
	72	4.89	34.8	46.6	16.4	37.0	2.91
	100	5.52	32.7	44.8	14.5	40.7	2.77
Pressure, lb./sq. in.	700	6.69	26.4	42.9	12.7	44.4	2.92
	1000	4.12	37.9	48.4	17.6	34.0	2.94
	1500	3.26	43.7	50.2	20.2	29.6	2.94

Increasing the absorption from 28 to 32% results in a decrease in opacity and percentage white and in an increase in percentages of black, yellow, and red; there is no change in the pigment content of the disks. Increasing the mixing time from 40 to 100 sec. increases opacity and percentage white, and decreases percentages of black, yellow, and red; there is also a definite decrease in pigment content. Increasing the pressure from 700 to 1500 lb. per sq. in. causes changes in the quality characteristics that are in exactly the same direction as those caused by increasing the absorption. It is interesting to note the relations that exist between colour characteristics and opacity: a decrease in opacity, whether caused by changes in absorption, mixing time, or pressure, is always associated with a decrease in the percentage of white and increases in the percentages of black, yellow, and red. The explanation for these relations appears to be relatively simple. As the disk becomes less opaque and thus more translucent, it reflects less total light, whether yellow, red, or white, and therefore requires a larger percentage of black in the matching disk. Moreover, larger percentages of the total light enter the paste to greater depths, and the reflected light is thus modified by a greater number of pigment molecules; the disks therefore appear yellower and redder respectively, the less opaque they are. These relations are best brought to light by reporting yellow, red, and white as percentages of the non-black component; they tend to be masked when the standard procedure of reporting all four colours as percentages required in the matching disk is adopted.

The pigment content of the disks requires separate treatment. It will be observed that it is unaffected by changing the absorption or pressure under which the disks are processed. On the other hand, with increasing mixing time there is a definite decrease in pigment content and this has been observed

in a number of other experiments. The authors are inclined to believe that the pigment is actually destroyed by oxidation during mixing. In this connection it will be recalled that the micro mixer (1) has a vigorous action: the paste is thoroughly mixed and many small air bubbles are therefore incorporated in it. It is clear, however, that this matter requires further investigation.

In view of the fact that the effects of processing factors on opacity have been discussed at some length in the preceding paper, and because changes in the colour characteristics are closely related to changes in opacity, it does not seem necessary to deal with the effects of processing factors on paste properties more extensively in this paper.

Interactions Between Processing Factors

The interactions between a considerable number of processing factors were also examined at some length in the preceding paper. Here again, however, the effects were examined only with respect to opacity. Accordingly, although it does not appear necessary to undertake detailed examination of the interactions between processing factors in this paper, it appears worth while to show that the interactions can be demonstrated not only with respect to opacity but also with respect to colour characteristics.

The matter can be considered most conveniently with the aid of results of analyses of variance. The pertinent portions of the analyses for each characteristic are recorded in Table IV. In this connection it should be pointed out, for those who are interested, that the complete analyses of variance can be obtained by combining data given in Tables II and IV; the error variance, representing the second and higher order interactions, is given in both tables.

It may be noted that the over-all effects of absorption on each quality characteristic are not significantly greater than the interaction between absorption and pressure. With respect to opacity, and percentage black, the same

TABLE IV

ANALYSES OF VARIANCE

Variance due to:	Degrees of freedom	Mean squares					
		Opacity coeff., K	Black segment	Non-black component			Pigment content
				Yellow	Red	White	
Absorption	2	7.12	72.1	40.8	14.7	96.8	0.003
Mixing time	2	32.38	579.0	258.2*	233.5**	982.8*	1.213**
Pressure	2	115.06*	2770.6*	524.0**	520.2**	2083.0**	0.004
Abs. × mixing time	4	0.05	2.3	3.6	2.1	9.9*	0.008*
Abs. × pressure	4	1.93**	55.6**	14.8**	12.3**	51.6**	0.002
Mix. × pressure	4	7.48**	119.3**	24.1**	12.9**	66.4**	0.006*
Error	68	0.07	4.0	1.5	1.2	3.0	0.002

Note: (*) and (**) denote that the 5% and 1% levels of significance are exceeded.

statement holds true for mixing time. On the other hand, the effect of pressure is significantly greater than the largest interaction involving pressure —that between pressure and mixing time—with respect to all quality characteristics except pigment content.

As was demonstrated in the previous paper, the interaction between mixing time and pressure is larger than that between absorption and pressure, and the latter is larger than the interaction between mixing time and absorption. This statement holds true with respect to all quality characteristics except pigment content. Moreover, the two larger interactions are significant to the 1% level with respect to both opacity and colour characteristics. On the other hand, the interaction between absorption and mixing time is only significant with respect to percentage white. In general, these findings are in accord with those of the previous paper; they serve to show that the general conclusions arrived at as a result of studying opacity only also apply to colour characteristics. It appears that the exact effect on past properties of a given change in one processing factor cannot be stated unless the levels at which all other processing factors were standardized are also specified. In other words, it may be inferred that, although a given change in pressure may have a marked effect on paste properties under certain processing conditions, if these conditions are changed, the effects of the same change in pressure may be greatly increased or decreased.

Pigment content deserves separate consideration. The analysis of variance shows that it is unaffected by changes in absorption and pressure, but that it is affected appreciably by changes in mixing time. Accordingly, it should be expected that only those interactions involving mixing time will be significant. The analysis of variance shows that this expectation is fulfilled; but it is clear that the interactions are extremely small, and are therefore of no practical significance.

References

1. CUNNINGHAM, R. L. and ANDERSON, J. A. Cereal Chem. 20: 171-185. 1943.
2. CUNNINGHAM, R. L. and ANDERSON, J. A. Cereal Chem. 20: 482-506. 1943.
3. FIFIELD, C. C., SMITH, G. S., and HAYES, J. F. Cereal Chem. 14: 661-673. 1937.

THE PROTEIN CONTENT OF CORRESPONDING GRADES OF WHEAT DRAWN FROM THE NORTHERN AND SOUTHERN PORTIONS OF WESTERN CANADA[1]

By J. Ansel Anderson[2] and William J. Eva[3]

Abstract

Data for 12 crops, 1927 to 1938, have been used to examine the protein contents of Grades 1, 2, and 3 Northern drawn from the northern and northwestern area, and from the central and southern area, of Western Canada. The boundary between the areas was taken as that dividing zones averaging over and under 13% protein. Grades 1, 2, and 3 Northern from the southern area averaged 14.2, 14.0, and 14.0% protein; those from the northern area averaged 12.8, 12.0, and 11.6% protein; and the average difference between zones for all three grades was 2.0%. The reasons for the increase in the spread between zones with decreasing grade are discussed. For the 12 yr. period, the average protein content of shipments of Western Canadian wheat is estimated as 13.5%. If no wheat had been shipped from the northern zone the average protein level would have been increased by about 0.5 per cent.

During the course of recent investigations the writers had occasion to calculate the mean protein levels for Grades 1, 2, and 3 Manitoba Northern drawn from the northern and from the combined central and southern part of Western Canada. The former area normally produces wheat of considerably lower protein content than the latter area. The difference between the average protein levels for the two areas was about 2%, but it was found that the difference was much greater for 3 Northern than for 1 Northern. It appeared worthwhile to collect additional information on these points, and the data for the 12 yr. period 1927 to 1938 have therefore been examined in some detail. The results are reported in this paper.

The study is of interest for several reasons. It throws light on the way in which current grade specifications affect the protein levels of different grades; it adds to information relating to the possibility of introducing protein content as a more direct grading factor; and it indicates the changes in the quality of export shipments of Western Canadian wheat that might be expected if wheat growing were confined largely to southern and central areas where the wheat of the best quality is normally produced.

The Data

The data available for the study consisted of 104,468 determinations of the protein content of Western Canadian wheat collected in annual surveys made by the Board of Grain Commissioners' Laboratory during the period

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1927 to 1938 (1). Of these 97,567 represented Grades 1, 2, and 3 Manitoba Northern. The first survey was made on a small scale with only 2726 samples from 1243 shipping points, but the project expanded rapidly. About 10,500 samples were collected from 1817 points in 1931, and over 12,000 samples were collected from 2094 points in 1932, the peak year. The average number of samples in each year was 8706, comprising 1571 from Manitoba, 4769 from Saskatchewan, and 2366 from Alberta; the average number of shipping points was 1772, comprising 353 in Manitoba, 930 in Saskatchewan, and 489 in Alberta.

When the surveys were started it was the intention to use the data only for mapping zones of different protein ranges. The method used in collecting the samples was admirable for this purpose and tended to produce the most accurate map possible at a reasonably early date in the fall of each year. However, the data contain a bias created by the method of collecting samples. The number of cars of wheat from each district, sampled for the survey in each year, was not proportional to the amount of wheat of the survey grades grown in or shipped from the district. Accordingly, too few samples were sometimes taken from low protein districts, and the mean values therefore tend to be higher than they should have been.

The lack of proportionality in sampling arose largely from two practical limitations placed on the survey collections. Firstly, in order to keep the total number of samples down to a reasonable figure, no more than eight samples were collected from any one shipping point; and secondly, in order to publish the data while it was of current interest, the survey was terminated early in November of each year. At that time, especially in certain years, comparatively little wheat had been shipped from many northern districts where the harvest was late, and it was thus difficult to obtain adequate numbers of samples from these normally low protein areas.

The nature of the bias in the data and its effect on various computed mean values have been discussed at some length in a previous paper (1), and it does not seem necessary to discuss the subject again. The writers are of the opinion that although the bias is sufficiently large to affect many of the mean values reported in this paper, it is not large enough to vitiate the general conclusions based on a study of the means.

Areas Designated as Northern and Southern Zones

For the purpose of the present study the crop-growing area in Western Canada was divided into a northern or low protein zone, and a remaining portion, which is subsequently referred to as the southern zone. The dividing line was taken as the boundary, on the 12 yr. mean protein map for the period 1927 to 1938, between zones in which the protein content of wheat averaged under and over 13%. Two small areas in southern Alberta representing stations that draw most of their wheat from irrigation districts were also included in the low protein zone. The two zones together with an approximate outline of the wheat-growing area are shown in Fig. 1.

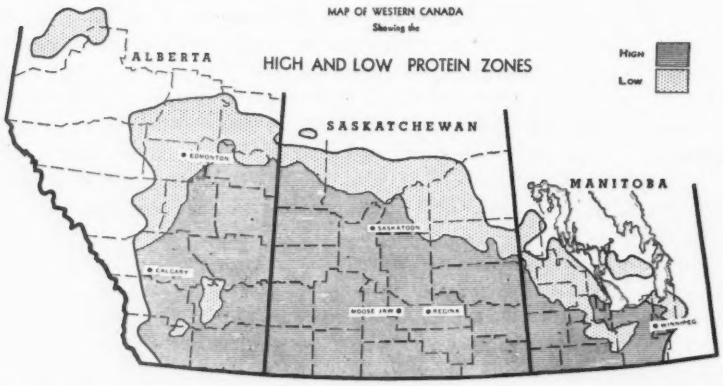

FIG. 1. *Map of Western Canada showing the high and low protein zones into which the wheat-growing area was divided for the study presented in this paper.*

Variations in the Protein Content of Carlot Shipments from Each Zone

In order to summarize and discuss the data within the limits of a comparatively short paper, it will be necessary to deal principally with the mean protein levels for the grades of different wheat crops drawn from different zones. These means, however, represent a major condensation of the data and therefore present only a limited picture of the conditions that actually exist in Western Canada. They tell nothing about the variations in the protein content of carlot shipments of the same grade drawn from the same zone, and it will be apparent that this is a matter of considerable interest.

These variations are illustrated by the distribution diagrams shown in Fig. 2. It did not seem desirable or necessary to reproduce diagrams for each of the 12 years. Accordingly, a low protein year, 1928, an average year, 1931, and a high protein year, 1936, were selected. To the diagrams for these years there has been added a set representing means for the 12 yr. period, 1927 to 1938. The solid line in each diagram shows the distribution of carlot shipments drawn from the southern zone, and the dotted line shows the corresponding distribution of samples drawn from the northern zone.

It must be pointed out that all diagrams represent the number of samples collected in the survey, rather than the amount of wheat shipped from the two zones. In general, the bias in the collection of samples resulted in too few being taken from the northern zone by comparison with the amount of wheat shipped from it. However, the error is not serious and if sampling had been proportional to shipments the diagrams for the low zones would have been only slightly larger than those shown in Fig. 2. It should also be noted that, as the number of samples of each grade collected in each year was not strictly proportional to shipments of the grades, the comparative areas of the diagrams in each column are not strictly indicative of the com-

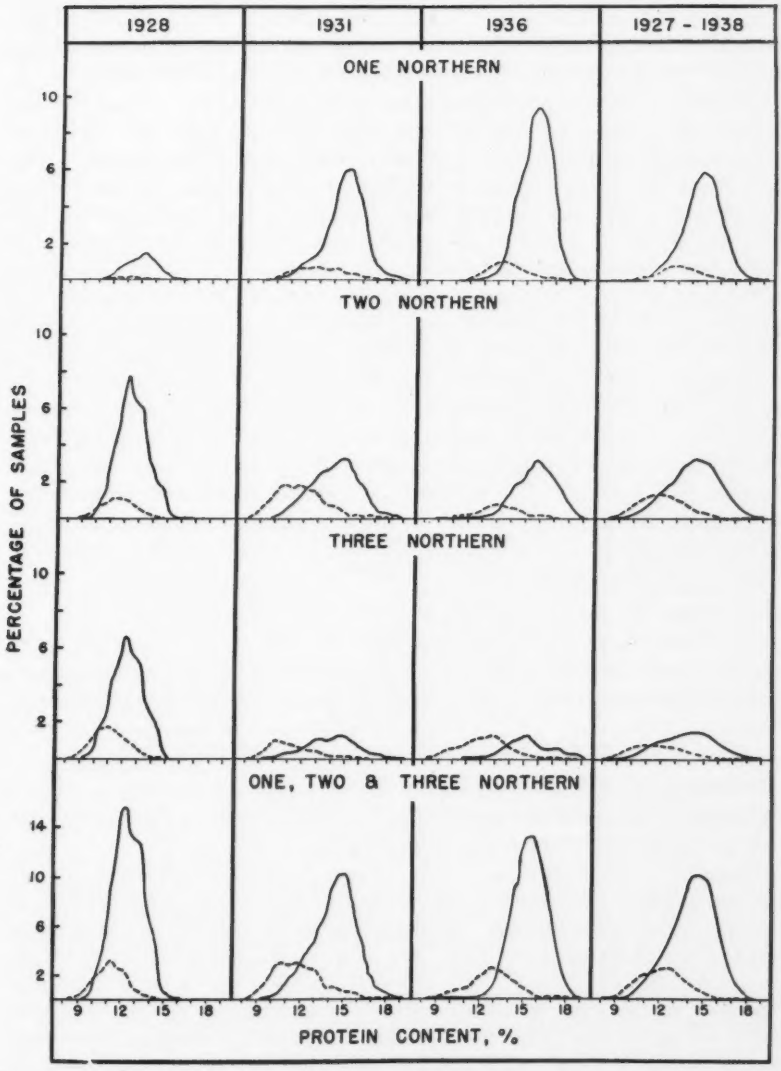

FIG. 2. *Diagrams showing the distribution over the protein range of samples drawn from the northern (dotted line) and southern (solid line) zones (cf. Fig. 1).*

parative sizes of the grades. Here again, however, a rough proportionality exists which is adequate for the purpose of this paper. Again, it must be noted that the diagrams for each crop are drawn on the percentage basis; thus each crop appears to be the same size, whereas the actual production

figures were 545 million bushels in 1928, 301 million in 1931, and 202 million in 1936. There appeared to be no advantage in making the diagrams for the different crops proportional in size to production.

Several features of the diagrams are at once apparent. Those for the low and high zones tend to cover essentially the same range, and the latter are moved only about one to two per cent towards the low protein side. The difference between the ranges for the two zones is least for the low protein year of 1928, intermediate for the average year, 1931, and greatest for the high protein year, 1936. This fact is apparent in the diagrams for the individual grades, but is illustrated best by the average diagrams for all samples shown in the bottom line of diagrams. When the three grades are compared on the basis of the 12-yr. mean diagrams, shown in the right hand column, it will be observed that the range for 1 Northern is slightly narrower than those for 2 and 3 Northern. Moreover, in the 1 Northern grade the range for samples from the low zone is not displaced so far to the left as in the other grades.

In view of the bias in the collection of survey samples, it is dangerous to draw attention to the comparative sizes and shapes of the various diagrams. Nevertheless, the writers, on the basis of wide experience with protein data for Western Canadian wheat, feel justified in drawing certain conclusions. On the average, the proportion of wheat in each grade, drawn from the low zone, increases with decreasing grade. The diagrams suggest that this occurs in each year, and the authors believe that this is generally the case. It also appears that when a grade contains a large proportion of the crop the distribution diagram is taller and has a sharper peak, whereas when the grade contains a small proportion of the crop the diagram tends to be broader and flatter.

Mean Protein Levels for Each Year and Each Zone

Bearing in mind information given in the last section, we may now turn our attention to the mean protein data which represent a more condensed and manageable summary of the data.

The mean protein contents of the survey samples collected from the high and low zones in each year are shown in the second and third columns of Table I. The fourth column contains the data for all samples, i.e., for both zones. It will be apparent that lack of proportionality in collecting the survey samples will affect all three sets of values. The writers have been unable to devise any method of testing the accuracy of the mean protein levels for each zone. However, general considerations suggest that these are not grossly in error. It is possible, however, to test the accuracy of the values for both zones combined. For this purpose the amounts of wheat of the survey grades shipped from low and high zones were determined for each year by reference to data on shipments from each railway division, published annually by the Dominion Bureau of Statistics. By accepting protein levels for each zone as accurate, and knowing the amount of wheat shipped from each zone, it was possible to estimate the mean protein content

TABLE I

MEAN PROTEIN LEVELS FOR EACH YEAR

Year	Protein content of samples, %*			Estimated protein level, both zones
	High zone	Low zone	Both zones	
1927	11.5	10.7	11.4	11.5
1928	12.4	11.4	12.2	12.3
1929	13.8	11.3	13.3	13.4
1930	13.6	11.3	13.1	13.2
1931	14.4	11.9	13.7	13.4
1932	14.4	12.2	14.0	13.9
1933	14.5	12.1	13.9	13.7
1934	14.7	12.0	14.1	13.8
1935	14.6	11.6	14.0	14.0
1936	15.5	12.7	14.9	14.7
1937	14.8	13.4	14.3	14.1
1938	14.3	12.9	14.0	14.0
Mean	14.0	12.0	13.6	13.5

* *All protein data ($N \times 5{\cdot}7$) are on a 13·5% moisture basis.*

of all wheat shipped from both zones. The resulting figures are given in the last column in Table I. It will be observed that in 1931 and 1934 the survey overestimated the protein level for Western Canada—i.e., both zones—by 0.3 per cent, and that an overestimation of 0.2 per cent occurred in 1933, 1936, and 1937. In all other years the difference between the protein content of samples from both zones and the estimated protein content is 0.1 per cent or less, which is negligible. On the whole, these data suggest that results of the surveys were not grossly in error.

The data in Table I indicate that, over the 12 yr. period, wheat from the high or southern zone averaged two per cent higher than wheat from the low or northern zone. The difference between zones varies considerably from year to year; it attained a maximum value of 3.0 per cent in 1935, and a minimum of 0.8 per cent in 1927. The average level for the high zone, 14.0%, represents a strong wheat with high supporting value, such as is required in export markets for blending with softer and cheaper wheats from other areas. On the other hand, the protein level of 12% for the low zone represents a filler wheat which would give little support to soft wheat mixtures.

It is also profitable to examine the difference between the estimated protein level for both zones and the level for the high zone. This difference represents the extent to which the average protein content of Western Canadian wheat would have been raised if no wheat had been shipped from the northern zone. The mean values given in the bottom row of figures in Table I show that an average increase of 0.5% would have occurred over the 12 yr. period. This can certainly be considered significant, and would be appreciated by most importers of Western Canadian wheat.

Mean Protein Levels for Each Grade

When the data are broken down by grades some interesting information is brought to light. The mean protein levels for each grade, over the 12 yr. period, from each zone and from both zones, are given in Table II. These are actual values obtained from the survey data; no method of correcting them for the bias in the collection of survey samples could be devised, other than one that required much more time than was available.

TABLE II

MEAN PROTEIN LEVELS, OVER ALL YEARS, FOR GRADES FROM HIGH, LOW, AND BOTH PROTEIN ZONES

Grades	Zones			High − low	High − both
	High	Low	Both		
1 Northern	14.2	12.8	14.1	1.4	0.1
2 Northern	14.0	12.0	13.5	2.0	0 5
3 Northern	14.0	11.6	13.2	2.4	0.8
Weighted mean	14.0	12.1	13.6	1.9	0.4

The differences between the levels for the high zone and both zones combined are of primary interest. They are shown in the last column of data in Table II. It is apparent that the difference increases with decreasing grade. The dissimilarity between grades results from a narrowing of the spread between the protein contents of the grades when wheat from the low protein zone is excluded. In other words, if wheat from the high zone were graded separately, the top three grades would tend to have essentially the same protein content and baking strength and would differ mainly in weight per bushel, flour yield, and percentage of damaged kernels.

In the low zone, the spread between the protein levels of No. 1 and No. 3 Northern is 1.2 per cent, as compared with 0.2 per cent for the high zone. There is thus a truly remarkable difference between the grades of the high and low protein zones; those from the former tend to be similar in protein content whereas those from the latter tend to be dissimilar.

It seems advisable to investigate next the extent to which this generalization holds within individual years. This is shown by the data on spreads and differences given in Table III. The spread between 1 and 3 Northern is smaller in the high zone than in the low zone in all years except 1937, when the spreads were equal in both zones, and 1938, when the spread was greater in the high zone.

An interesting feature of these data concerns the spreads in the high zone for the years 1932, 1933, 1934, and 1935. These are marked with a negative sign in the table since in these years the protein level for 3 Northern was higher, rather than lower, than the level for 1 Northern. It will be apparent

TABLE III

COMPARISON OF THE PROTEIN CONTENTS OF GRADES 1, 2, AND 3 NORTHERN FROM EACH ZONE AND FOR EACH YEAR

Year	Spreads between 1 and 3 Northern			Differences between high and low zone		
	High zone	Low zone	Both zones	1 North.	2 North.	3 North.
1927	0.7	0.8	0.7	0.7	0.2	0.8
1928	0.5	1.1	0.7	0.5	0.8	1.1
1929	0.1	1.2	0.8	1.6	2.4	2.9
1930	0.8	1.3	1.4	1.8	1.9	2.3
1931	0.6	1.5	1.4	1.7	2.4	2.6
1932	−0.4	1.3	0.2	1.4	2.6	3.1
1933	−0.6	1.7	0.4	1.4	2.8	3.7
1934	−0.6	1.7	0.7	1.5	3.1	3.8
1935	−0.4	1.0	1.1	2.1	2.3	2.7
1936	0.2	1.5	1.7	1.9	2.4	3.2
1937	0.5	0.5	0.9	1.3	1.7	1.3
1938	0.4	0.2	0.6	1.5	1.2	1.3
Mean	0.2	1.2	0.9	1.4	2.0	2.4

that these negative values could not be disregarded in calculating the mean spread for the high zone shown in Table II and in the bottom line of Table III.

The last three columns of data in Table III show the differences between high and low zone means for each grade and each year. In almost all years the difference between zone means increases from 1 Northern to 2 Northern and again from 2 Northern to 3 Northern. Thus the conclusions drawn from the summarized data in Table II are again supported.

In Table IV the data are summarized by provinces. In each province the spreads between the protein contents of 1 and 3 Northern are considerably greater in the low than in the high zone. It will be noted, however, that in this respect Manitoba shows the least difference between zones, presumably because of the small size of the crop-growing area in that province. In all three provinces the difference between protein levels for high and low zones increases with decreasing grade; but here again the increase is least in Manitoba. The last column of data show the effect on the protein content of each grade of removing the wheat grown in the low protein zone. The increases in protein content are actually greatest in Alberta, as would be expected since that province produces a larger proportion of its wheat in the low protein zone than do Saskatchewan and Manitoba. In general the study of the provincial summaries, given in Table IV, fully supports the conclusions based on summarized data representing Western Canada as a whole.

Discussion

The causes of the variations that occur from district to district, and from year to year, in the protein content of Western Canadian wheat, have been discussed at some length in a previous publication (1). In the present paper

TABLE IV

MEAN PROTEIN CONTENTS, OVER ALL YEARS, FOR GRADES FROM HIGH, LOW, AND BOTH PROTEIN ZONES, IN EACH PROVINCE

Province	Grade	Zones			Differences	
		High	Low	Both	High – low	High – both
Manitoba	1 Northern	13.8	12.7	13.4	1.1	0.4
	2 Northern	13.5	12.3	13.0	1.2	0.5
	3 Northern	13.4	12.0	12.8	1.4	0.6
	Spread	0.4	0.7	0.6		
	Weighted mean	13.6	12.4	13.2	1.2	0.4
Saskatchewan	1 Northern	14.3	12.7	14.2	1.6	0.1
	2 Northern	14.2	11.9	13.7	2.3	0.5
	3 Northern	14.3	11.8	13.6	2.5	0.7
	Spread	0.1	0.9	0.6		
	Weighted mean	14.2	11.9	13.8	2.3	0.4
Alberta	1 Northern	14.2	12.7	14.0	1.5	0.2
	2 Northern	14.0	11.9	13.3	2.1	0.7
	3 Northern	13.8	11.4	12.6	2.4	1.2
	Spread	0.4	1.3	1.4		
	Weighted mean	14.0	11.8	13.3	2.2	0.7

discussion will therefore be confined mainly to the differences that occur between high and low zones with respect to the spreads between the protein levels of corresponding grades. In the southern zone the existing grading procedures show little tendency to create a spread in protein content between 1 and 3 Northern, whereas in the northern zone an appreciable spread occurs almost invariably. This seems surprising in view of the fact that the distribution diagrams show that the wheat from each zone covers essentially the same protein range. The explanation must be that grading factors now in use in Canada are more highly correlated with protein content in the north than in the south. In order to examine this hypothesis the various grading factors are considered individually in the following paragraphs.

The grading factors for hard red spring wheat, as outlined in Schedule One of the Canada Grain Act, are:— minimum weight per measured bushel; variety; minimum percentage of hard vitreous kernels; degree of soundness; and maximum limits of foreign materials, for which limits are set both for wheat of other classes or varieties, and for foreign materials other than wheat.

It is well known that weight per bushel is appreciably higher in the low protein zone than in the high zone. This fact is amply established by data published each year in the Annual Report of this laboratory, which show that Edmonton, the office that draws the greatest proportion of its wheat from the low zone, invariably shows a higher average for weight per bushel of hard red spring wheat than any other inspection office in the Western Division. Further data bearing on this point were obtained in the survey of carlot shipments for the 1940–41 crop year. It was found that weight per bushel

averaged 62.7 lb. in the low protein zone as compared with 61.6 lb. in the high zone. Thus a difference of over 1 lb. per bushel existed in favour of the low zone in 1940, which was a year of high yields when weight per bushel tended to be better than usual in the central and southern sections covered by the high protein zone. Thus it would appear that weight per bushel must operate to a greater extent as a degrading factor in the south, or high protein zone, than in the north.

Bushel weight tends to be inversely correlated with protein content, but the relation is by no means close. It follows that the segregation of wheats into grades, if based only on bushel weight, tends to create an inverse correlation between grade and protein content. The use of bushel weight as a grading factor therefore serves to offset the effects of other grading factors that operate to decrease protein content with decreasing grade. As weight must be taken into account more frequently in grading southern wheat, it follows that a narrowing of the spreads in protein content between grades resulting from this cause must also occur more frequently in the south. Indeed, in some years, when there is little damage to the crop, bushel weight becomes the only important grading factor and under such circumstances protein content may actually increase with decreasing grade. The data in Table III show that this situation occurred in the high zone in four of the 12 years; it did not occur in any year in the low zone. The use of weight per bushel as a grading factor therefore appears to offer a partial explanation of the differences between the grade spreads in the two zones.

It is doubtful whether differences between the varieties grown in the low and high zones have any appreciable effect on the matter under discussion. Garnet, the only variety grown mainly in the north, was not produced in quantity prior to 1935, and was graded separately and excluded from Grades 1, 2, and 3 Northern from 1935 on. According to the Act, No. 1 and No. 2 Northern must contain only Marquis or varieties "equal to Marquis", while No. 3 Northern may contain varieties of red spring wheat of fair milling quality, excluding Garnet. In order for differences in variety to affect the spread in protein contents between 1 and 3 Northern, it would be necessary to assume not only that a greater percentage of wheat of varieties not "equal to Marquis" was grown in the low zone, but also that these varieties tended to be lower than Marquis in average protein content. There is no evidence suggesting that this situation occurred.

Turning to the third grading factor, minimum percentage of hard vitreous kernels, we find another reasonable hypothesis. The minimum percentages required in the top three Northern grades are 65, 50, and 35%, respectively. It must be remembered that the inspector, in grading wheat, must determine at a glance the approximate percentage of hard vitreous kernels, and decide whether a count is necessary to determine whether the wheat should be degraded for failure to meet the minimum specifications. In any event the matter must be decided wholly on the appearance of the kernels. Now it is well known that soft, or "yellow-berry", kernels do not normally appear in

wheat having a protein content of over about 13%. While some differences in apparent vitreousness may exist in samples averaging over 13% in protein, those with the higher protein content tending to look somewhat more vitreous, almost all kernels in such samples must normally be classed as vitreous. On the other hand, in samples of lower protein content, kernels that must obviously be classed as soft appear more frequently, and a moderate degree of correlation exists between the percentage of vitreous kernels and protein content (2, and papers cited therein). It is clear that minimum percentage of hard vitreous kernels must be applied as a direct grading factor much more frequently in the low protein zone, where the wheat averaged 12% protein, than in the high zone, where the average protein level was 14%. In the northern zone there is a greater probability of encountering low protein samples, and, since these can be recognized and degraded for lack of vitreousness, the spread in protein level between 1 and 3 Northern is increased. In the southern zone this grading factor is applied only infrequently and has less effect on the spread in question.

Degree of soundness also appears to be an important factor in relation to the matter under discussion. Wheat grading No. 1 Northern must be well matured and practically free from damaged kernels; No. 2 Northern wheat must be reasonably well matured and reasonably free from damaged kernels; while No. 3 Northern may contain wheat excluded from higher grades on account of lightly frosted, immature, or other light damage, and must be reasonably well matured. The inclusion in the specifications for No. 3 Northern of references to lightly frosted and immature kernels gives the key to the types of damage that deserve closest attention.

In the low zone, which covers the northern and western borders of the crop-growing area, the growing season is shorter, and immature, frosted, bleached, sprouted, and weathered grains therefore tend to be considerably more prevalent. Accordingly, much larger quantities of grain are degraded for these types of damage in the low zone than in the high zone. It remains to consider whether these grading factors, associated with the degree of soundness, are also associated with protein content in the low zone. There is good reason to believe that they are. The mean protein map for Western Canada (1) shows that the further north wheat is grown the lower its protein content tends to be, and it is also apparent that the further north wheat is grown the greater the chance of its being cut on the immature side or damaged by frost and similar factors. Moreover, both immature and frosted kernels are more likely to be found in wheat grown on low land than on high land, and the wheat from the low land also tends to be lower in protein content than that from the high land. An inverse correlation between protein content and damage must therefore be expected. As such degrading factors as immaturity, frost, and weathering are more frequently applied in the northern than in the southern zone, they must also tend to cause a greater spread in the north than in the south between the protein contents of 1 and 3 Northern.

Maximum limits of foreign materials do not appear to affect the matter under consideration. It is true that mixed farming is much more widely practised in the north than in the south and that there is thus a greater danger in the north of mixing small amounts of oats or barley with the wheat. This might result in some degrading since 1 Northern must be practically free from these other grains, while 2 Northern may contain 1% and 3 Northern may contain 2%. A slight but definite tendency towards lower protein content would occur in lower grades containing small amounts of barley and oats, merely because these grains when grown side by side with wheat are almost invariably lower in protein content. However, this factor would not have affected the data presented in this bulletin since all protein analyses were made on cleaned wheat from which all other grains had been removed.

Consideration of the points put forward in the preceding discussion will show that there are several good explanations of the fact that the spread in protein content between 1 and 3 Northern is greater in the low protein zone than in the high protein zone. Since this difference in spread also involves an increasing difference between the high zone and low zone protein levels with decreasing grade, it follows that this fact is also susceptible of ready explanation.

As this paper is being written, it is the policy in Canada to reduce wheat acreage by encouraging the production of other grains. Whether it will be necessary to continue this policy after the war remains to be seen, but the possibility cannot be entirely discounted. If smaller wheat acreage is desirable, there are many who contend that the reduction should take place mainly in the northern and northwestern areas. It is argued that these areas produce wheat of poor quality, but are well suited to mixed farming, whereas many parts of the central and southern area are best suited to grain farming and produce wheat of excellent quality in almost all years.

The data presented in this paper illustrate the probable effect of restricting northern wheat acreage. If no wheat were grown in the low protein zone outlined in Fig. 1, an increase of about 0.5 per cent would occur in the average protein content of Western Canadian wheat. This would be associated with an average decrease in the spreads between the protein contents of Grades 1, 2, and 3 Northern, and in most years these grades would have essentially the same protein content. It is the writers' opinion, though little data can be produced to support it, that,there would also be changes in bushel weight. The average weight per bushel of the Western Canadian crop would probably drop by 1 to 1.5 lb., and the spread in weight between the grades would probably increase. Some change in the appearance of average samples of the lower grades might also occur. These grades would tend to be more uniform in appearance in certain years. At present, both 3 and 4 Northern may contain mixtures of wheat degraded merely because of lower bushel weight and of plump wheat degraded for immaturity or frost damage. Since the latter wheats generally come from the north a considerable reduction in

the amounts of these types entering Grades 3 and 4 Northern would be expected.

It seems certain that these changes would be appreciated by most of the overseas importers of Canadian wheat. The wheat is bought mainly for its high baking strength, and this would be increased, particularly in the lower grades. The gain would be offset, in part, by a decrease in bushel weight and flour yield, but this would also occur mainly in the lower grades. Any increase in uniformity within grades is, of course, highly desirable both from the viewpoint of the buyer and from that of the seller.

It is unfortunate that precise information on the bushel weights of wheat from the northern and southern zones is not available. Steps have been taken to collect the data, and when a sufficient number have accumulated it will be possible to investigate the matter.

References

1. ANDERSON, J. A. and EVA, W. J. Grain Research Lab., Board of Grain Commissioners for Canada, Winnipeg, Bull. 4. 1943.
2. BAILEY, C. H. Minnesota Agr. Expt. Sta., Tech. Bull. 147. 1941.

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DRIED WHOLE EGG POWDER

VI. EFFECT OF STORAGE TEMPERATURE AND GAS PACKING ON KEEPING QUALITY[1]

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Abstract

Dried whole egg powders were obtained from three different manufacturers and stored at temperatures ranging from 7.1° to 32.1° C. for periods up to six months. Quality was assessed by determination of fluorescence and potassium chloride values. At 23.8° C. the rate of deterioration was comparatively rapid; at 32.1° C. it was markedly so. To maintain quality during storage and transport dried egg should be stored at a temperature of 15.6° C. or lower.

The effect on keeping quality of packing in nitrogen, carbon dioxide, *in vacuo*, or in the form of compressed tablets was studied. Carbon dioxide alone had beneficial effect.

Introduction

In a recent investigation it was shown that the quality of dried whole egg powder was materially affected by the rate at which the powder was cooled after drying (3). The observed thermal instability of egg powder indicated that a study of suitable conditions for storage and transport was necessary. The present paper deals with the effect of temperature, gas packing, and compression on the keeping quality of dried whole egg powder during storage.

Materials and Procedure

Storage Temperature

Egg powder was obtained from three representative Canadian egg drying establishments. The powder from Plant I was prepared from a mixture of 95% Grade A and 5% Grade B fresh eggs dried in a box-type drier at inlet and outlet temperatures of 150° and 58° C. (302° and 137° F.) respectively; that of Plant II from a 1:1 mixture of Grade A and B fresh eggs dried in a modified box-type drier at inlet and outlet temperatures of 108° and 59° C. (227° and 138° F.) respectively; and that of Plant III from Grade A fresh eggs at inlet and outlet temperatures of 110° and 51° C. (230° and 123° F.) respectively.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. A portion of the material was selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as paper No. 96 of the Canadian Committee on Food Preservation and as N.R.C. No. 1140.

[2] *Biochemist, Food Investigations.*

Samples of powder (100 gm.) from each of the three plants were placed in cardboard containers with metal ends and stored at temperatures of 7.1°, 15.6°, 23.8°, and 32.1° C. (45°, 60°, 75°, and 90° F.). The use of this type of container for storage was prompted by the present extensive application of "ersatz" containers in commercial practice. Samples were removed for analysis after storage for one, two, four, and six months at 7.1°, 15.6°, and 23.8° C. and at semimonthly intervals for a total period of three and one-half months at 32.1° C. Changes in quality were assessed by determination of the fluorescence and potassium chloride values (1, 2).

Gas Packing

The dried whole egg powder used in the gas-packing studies was similar to that secured from Plant II for the storage experiment. Five different methods of packing were studied, namely, in air, carbon dioxide, or nitrogen; under vacuum; and compressed in the form of a 100-gm. tablet. Samples to be packed in gas or *in vacuo* were placed in glass flasks, thoroughly evacuated with a Hyvac pump, flushed three times with gas where desired, and the flasks sealed off. The tablets were compressed under 800 lb. per sq. in. at room temperature. Pressure was increased rapidly to the desired level and released immediately to minimize exudation of egg oil. The tablets and air-packed material were both stored in sealed tin cans.

Duplicate samples were removed for analysis after storage for one, two, and six months at 23.8° C. (75° F.). Quality of the powders was assessed by determination of the fluorescence and potassium chloride values (1, 2).

Results

Storage Temperature

The effect of storage temperature, storage time, and source of powder on quality may be seen from the mean fluorescence and potassium chloride values given in Table I. Differences with temperature were greatest between 23.8° and 32.1° C. and least between 7.1° and 15.6° C. The changes in mean fluorescence values with time for powders stored at 7.1° to 23.8° C. were greatest between the two-, four-, and six-month periods, whereas at 32.1° C. the values increased rapidly during two and one-half months and subsequently remained essentially at the same level. Similarly the potassium chloride values showed marked decrease between two and four months at 7.1° to 23.8° C. and during the first one and one-half months in storage at 32.1° C.

The relative order of the mean fluorescence and potassium chloride values for the three plants was the same after storage as observed initially. However, the magnitudes of the differences between plants, in terms of mean fluorescence values of the powder, were slightly smaller after storage, while those in mean potassium chloride values were larger. Thus, greater changes occurred during storage in powders of low than of high fluorescence values. The opposite behaviour was observed for the potassium chloride values except

TABLE I

MEAN FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS AFTER STORAGE FOR VARIOUS PERIODS AT 7.1° TO 32.1° C.

Temperature

Temperature, °C.	7.1	15.6	23.8	32.1
Mean fluorescence value [1, 2]	27.5	29.2	33.7	49.6
Mean KCl value [1, 2]	66.5	63.4	54.6	41.5

Storage period

Time, months	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	6.0
Mean fluorescence value[1]	28.1	–	27.3	–	26.4	–	–	–	30.5	36.3
Mean fluorescence value[2]	28.1	38.7	40.2	49.3	55.1	62.7	62.9	59.5	–	–
Mean KCl value[1]	71.4	–	69.9	–	68.2	–	–	–	54.2	53.8
Mean KCl value[2]	71.4	42.0	67.6	33.1	31.8	29.6	29.2	26.8	–	–

Plant

Plant	I	II	III
Mean fluorescence value[1]	36.0	28.8	25.6
Mean fluorescence value[2]	51.8	50.1	46.8
Mean KCl value[1]	53.2	66.4	65.0
Mean KCl value[2]	38.5	44.9	41.0

[1] *Mean values over all other conditions studied for powder stored at 7.1°, 15.6°, and 23.8° C.*
[2] *As for [1], but on powder stored at 32.1° C. only.*

at 32.1° C. where the differences between plants were about the same as they were initially. This point will be discussed further in a subsequent paper.

Analyses of variance were used to assess the relative importance of the factors studied. Since the sampling times employed at 32.1° C. differed from those at the lower temperatures, these data were treated separately. The source of the powder and temperature and time of storage all had highly significant effects on both the fluorescence and potassium chloride values except at 32.1° C. where the differences in potassium chloride values between plants failed to reach the level of statistical significance (Table II).

Of the differential quantities, that relating temperature with storage period was alone significant. Details of the changes in fluorescence values during storage at 7.1° to 23.8° C., and at 32.1° C. are given in Tables III and I respectively. The fluorescence values for powders stored at 7.1° and 15.6° C. were essentially unchanged after four months. In this connection it is

TABLE II

ANALYSES OF VARIANCE OF FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1° to 32.1° C.

Storage temperature, °C.	Source of variance	D.f.	Mean square	
			Fluorescence value	KCl value
7.1°, 15.6°, and 23.8° C.	Temperature	2	249.2**	917.7**
	Time	3	366.3**	1366**
	Plants	2	679.7**	1268**
	Stored vs. non-stored	1	21.97*	544.8**
	Temperature × time	6	21.02**	160.7*
	Temperature × plants	4	0.19	45.06
	Residual	20	4.27	
	Duplicate error	39	0.50	3.20
32.1° C.	Time	6	619.7**	1219**
	Plants	2	100.1*	166.3
	Stored vs. non-stored	1	3146**	6171**
	Residual	14	21.10	91.03
	Duplicate error	24	1.514	1.21

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

probable that the values for four and six months' storage at 7.1° C. are somewhat higher than would normally be obtained since the moisture content of the powders increased from five to about seven per cent in this period. It has recently been shown that moisture content has considerable effect on the keeping quality of egg powder (4). The reason for the apparent decrease in the fluorescence values after one and two months is unknown. Since the fluorescence values increased at a relatively rapid rate at 23.8° C. and markedly so at 32.1° C., it is apparent that egg powder should be held at a temperature of 15.6° C., and preferably lower, if quality is to be maintained during storage and transport.

TABLE III

FLUORESCENCE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1°, 15.6°, AND 23.8° C.

Time, months	Temperature, °C.		
	7.1	15.6	23.8
1	26.0[1]	26.9[2]	28.8
2	23.7	24.5	30.9
4	29.0	29.7	33.0
6	31.3	35.5	42.2

[1] *All values as the means for three plants.*
[2] *Necessary difference to exceed 5% level of statistical significance: 2.5.*

While the potassium chloride values of egg powder decreased during storage at all temperatures, the changes were least and approximately of the same magnitude at 7.1° and 15.6° C. (Tables IV and I). This confirms the conclusions based on fluorescence values, and emphasizes the necessity for storing whole egg powder at temperatures of 15.6° C. or lower.

TABLE IV

POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1°, 15.6°, AND 23.8° C.

Time, months	Temperature, °C.		
	7.1	15.6	23.8
1	71.9	67.1	70.8
2	71.4	70.7	62.4
4	61.0	57.9	43.6
6	61.8	58.1	41.6

[1] *All values as the means for three plants.*
[2] *Necessary difference to exceed 5% level of statistical significance: 8.0.*

Gas Packing

The effect of the various methods of packing studied on the fluorescence and potassium chloride values are given, together with analyses of variance, in Tables V and VI. Packing in carbon dioxide had definite preservative effect, as indicated by fluorescence and especially by potassium chloride values. Storage in nitrogen, under vacuum, or in the form of a compressed tablet apparently had little effect. The suitability of gas packing with carbon dioxide is being given further study.

TABLE V

EFFECT OF VARIOUS METHODS OF PACKING ON THE FLUORESCENCE VALUES OF DRIED EGG POWDER STORED AT 23.8° C.

Mean values

Storage period, months	Method of packing				
	Air	Vacuum	Carbon dioxide	Nitrogen	Tablet
1	25.5[1]	27.6	25.5	28.9	28.7
2	29.2	31.5	24.8	31.2	28.8
6	44.5	61.4	38.6	46.5	42.4

Analysis of variance

Source of variance	D.f.	Mean square
Method of packing	4	179.9**
Time	2	2299**
Method of packing × time	8	82.37**
Duplicate samples	15	5.352
Duplicate analyses	30	0.8588

[1] *Necessary difference to exceed 5% level of statistical significance: 3.5.*
** *Indicates 1% level of statistical significance.*

TABLE VI

EFFECT OF VARIOUS METHODS OF PACKING ON THE POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDER STORED AT 23.8° C.

Mean values

Storage period, months	Method of packing				
	Air	Vacuum	Carbon dioxide	Nitrogen	Tablet
1	64.3[1]	66.2	71.6	70.4	61.8
2	59.8	56.3	73.2	55.8	57.8
6	39.1	37.8	72.2	51.6	40.7

Analysis of variance

Source of variance	D.f.	Mean square
Method of packing	4	783.7**
Time	2	1786**
Method of packing × time	8	165.0
Duplicate samples	15	8.714
Duplicate analyses	30	1.844

[1] *Necessary difference to exceed 5% level of statistical significance: 4.5.*
** *Indicates 1% level of statistical significance.*

Acknowledgments

The authors wish to express their thanks to Mr. T. A. Steeves, Refrigeration Engineer, for his assistance in establishing and maintaining the storage conditions, and to Mr. L. Moore, Laboratory Assistant, for his technical assistance.

References

1. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20: 276-282. 1942.
2. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
3. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.
4. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.

THE DEVELOPMENT OF THE WILD TYPE AND BAR EYES OF *DROSOPHILA MELANOGASTER*[1]

By Arthur G. Steinberg[2]

Abstract

The histological development of the eyes of Bar and wild type *Drosophila melanogaster* during the larval and pupal stages is described. The onset of morphological differentiation of the ommatidia of both Bar and wild type occurs between 48 and 72 hr. after hatching from the egg (temperature = 25° ± 0.2°C.). At all stages the developing ommatidia of Bar and wild type are the same. At no time was evidence of degenerating ommatidia found in any region of the Bar eye. The data are discussed in the light of several hypotheses that have been advanced to explain the development of the Bar eye.

Introduction

When Drosophila geneticists turned to the study of the role of the gene in development, they were, and in fact still are, repeatedly handicapped by a lack of knowledge of the normal development of this organism. Before Poulson could satisfactorily study the effect of various deficiencies on the development of the embryo (16) he was compelled to do a pioneer study of the normal ontogeny of the early embryo (15). Kaliss (9) in his study of the development of the yellow achaete deficiency found it necessary to describe the normal ontogeny of the late embryo; the situation is similar with regard to studies on the wings and limbs (1, 5, and 23).

Although the eye is probably the organ most studied by those interested in developmental genetics, and many theories concerning the mode of action of at least one mutant (Bar) on its development have been elaborated, relatively little is known about its development. (This may be an important reason for the many theories elaborated to explain the action of the Bar mutation.) The few studies of the histological development of the eye that have been made have led to contradictory conclusions. A brief survey of these studies follows.

The time of onset of the histological differentation of the ommatidia has been reported by Krafka (10) and Chen (3) to be 24 hr. before pupation (temperature not given). Medvedev (12) reported that the "ommatidia" can be seen in stained whole mounts of optic disks of mature larvae but made no mention of studies of earlier stages. On the other hand Bodenstein (2) and Robertson (11) claim that the ommatidia do not begin to differentiate until at least 36 hr. after puparium formation. In contrast to the above reports Enzmann and Haskins (4) state that they observed the onset of ommatidial differentation as early as 18 hr. after egg deposition. The author (19) has already reported that he was unable to confirm Enzmann and Haskins' obser-

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vations. Recently Pilkington (14) reported the results of studies of the histological development of the normal and some mutant eyes. He claimed that up to "the four day larva the optic disc (consists) of a mass of cells several layers thick but apparently homogenous." This claim is certainly not so as shown by the present author (19) and also Krafka (10). Pilkington's detailed description of the subsequent development of the eye will be reviewed below.

The resolution of these contradictions became particularly urgent to the present author when his study of the growth curves of wild type, Bar, and modified Bar eye disks (19 and 20) led him to conclude that the reduced number of facets exhibited by the Bar eye was the result of a reduction of the number of cells entering into the formation of the cephalic complex; it was also postulated that the range of facet number over which an eye is able to vary is determined by the presence of a number of cells that are labilely determined to form facets or head chitin, depending upon the action of extrinsic and intrinsic factors (19 and 20).

Preliminary histological examination of wild type eye disks showed that in the wild type the cells of the optic disk begin to organize into "clusters" of four cells each at about 72 hr. after hatching from the egg at 25° C. The "clusters" increased greatly in both number and clarity by 96 hr. after hatching (19). However, these findings could not be confirmed for Bar at that time; i.e., no organization of cells into larger units was observed.

The questions left open therefore were (*a*) when, if at all, do these "clusters" arise in the Bar eye disk and (*b*) what is the subsequent role of these "clusters" in the development of the eye?

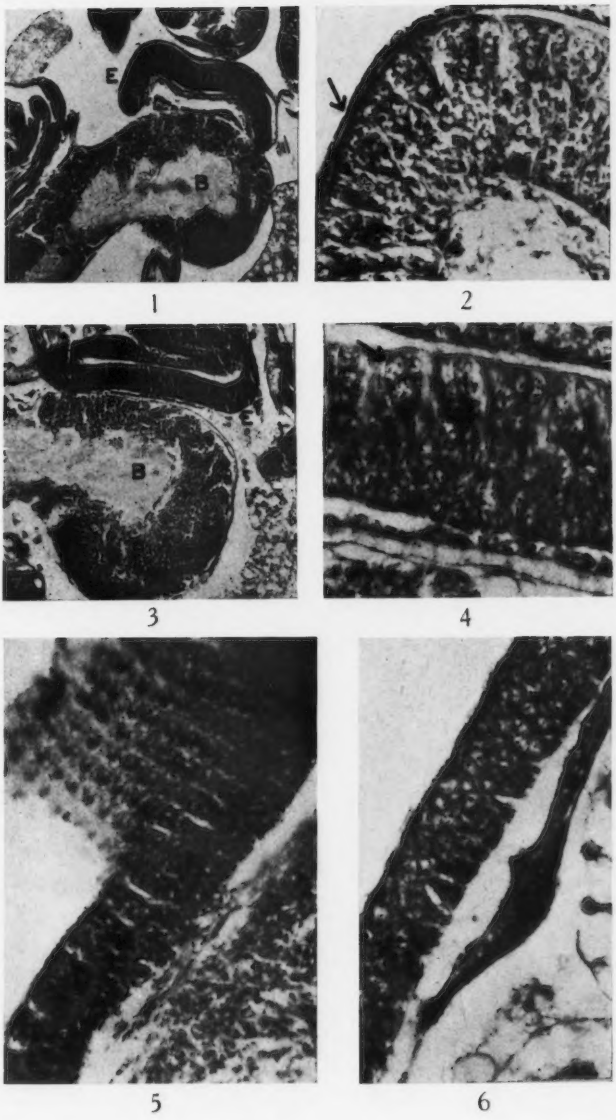
This paper is a report of studies undertaken to obtain answers to these questions. An abstract of part of the data discussed below is published in *Genetics* (21).

Material and Methods

The stocks of *Drosophila melanogaster* used for these experiments were (*a*) an inbred Florida wild type and (*b*) an inbred Bar that had been rendered isogenic with the Florida strain by repeated backcrossing. Both these stocks had been inbred for more than 100 generations by the beginning of these experiments.

All larval ages were determined from the time of hatching and were accurate to within one hour. The larvae were raised in 3-in. Petri dishes (20 to 30 larvae per dish) on a food medium consisting of 2% agar plus 12.5% of molasses in water, seeded 24 hr. before use with two drops of a heavy yeast suspension.

All pupal ages were determined from the time of puparium formation. The onset of puparium formation was taken as the time when the larva was no longer capable of moving its anterior spiracles in response to a stimulus. For the sake of simplicity of discussion, both prepupae and pupae will be referred to as pupae (true pupation occurs 12 hr. after puparium formation



All prints are contact prints. All magnifications given are initial magnifications at the ocular. B = brain; E = eye disk.

FIG. 1. *Wild type larva 96 hr. after hatching. 140×.*

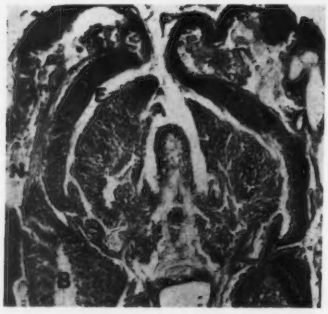
FIG. 2. *High power photograph of a portion of the eye disk shown in Fig. 1. 840×. Several "goblets" may be seen. The arrow points to a particularly clear one.*

FIG. 3. *Wild type "pupa" five hours after puparium formation. 140×.*

FIG. 4. *High power photograph of a portion of the eye disk shown in Fig. 3. 840×. The arrow indicates one of five "goblets" shown.*

FIG. 5. *Section through an eye disk of a six hour wild type pupa. 600×. Because the disk was curved the ommatidial precursors were cut in cross section in one portion ("clusters" at upper left) and in longitudinal section in another ("goblets" at lower right).*

FIG. 6. *Longitudinal section through the eye of a 12 hr. wild type pupa. 840×.*



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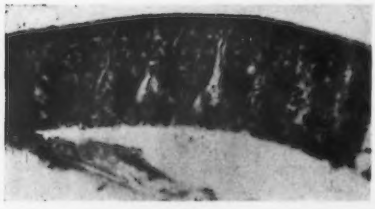

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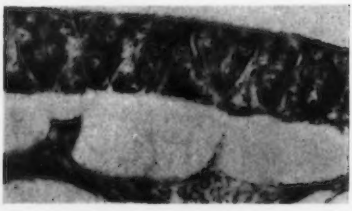

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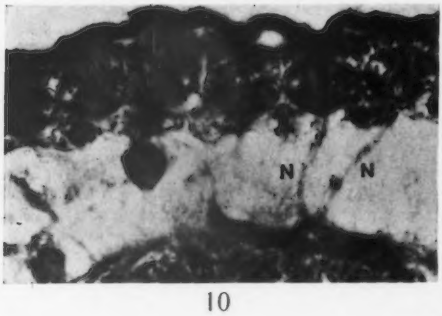

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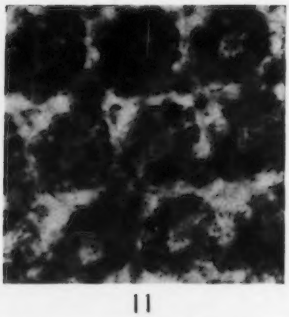

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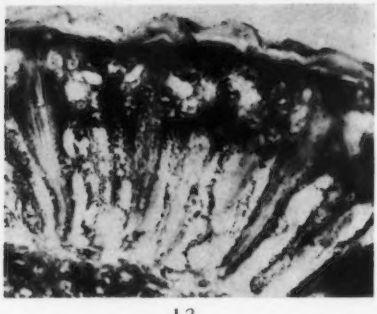

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All prints are contact prints. All magnifications given are initial magnifications at the ocular. A = antennal disk; B = brain; E = eye disk; N = optic nerve.

FIG. 7. *Frontal section through a 10 hr. wild type pupa. 140 ×. Note the position of the optic nerve.*

FIG. 8. *Longitudinal section through the eye of an 18 hr. wild type pupa. 840×.*

FIG. 9. *Longitudinal section through the eye of a 24 hr. wild type pupa. 840×.*

FIG. 10. *Longitudinal section through the eye of a 48 hr. wild type pupa. 840×.*

FIG. 11. *Cross section through the eye of a 48 hr. wild type pupa. 840×.*

FIGS. 12 AND 13. *Longitudinal section through the eye of a 72 hr. pupa. 140× and 840×, respectively.*

at 25° C.). Throughout these experiments the temperature used was 25 ± 0.2° C.

Previous experience (19) had shown that best histological results were obtained by fixing at 50° C. for one-half to one hour in a modified Carnoy mixture consisting of glacial acetic, two parts; chloroform, three parts; and absolute alcohol, five parts. Consequently all fixations were done with this solution. Both larvae and pupae were sectioned at 10 μ and stained in Delafield's haematoxylin with eosin as a counterstain.

Data

Wild Type

Re-examination of the larval eye disks at 48, 72, and 96 hr. after hatching served to confirm the author's previously reported observations (19). There is no organization of the cells into units of a higher order before 72 hr. of larval life. The units then formed are few in number and are seen to consist of clusters of four cells each (however, see below). These "clusters" increase greatly in number by 96 hr. They can be readily seen in whole mounts lightly stained in eosin, as reported by Medvedev (12), Krafka (10) and others, including the present author. These observations in no way coincide with Pilkington's (14) diagrams of the eye disks of mature larvae (compare Figs. 2, 4, 15, and 17 of this paper and his Text Fig. 1A). As a matter of fact his diagrams of subsequent stages cannot at all be reconciled with the present author's observations. This is clearly brought out by a comparison of Pilkington's Text Fig. 1 and the photographs in Plates I and II of this paper.

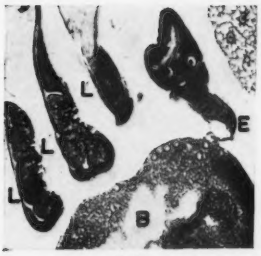
Among the first slides examined of pupal stages was one of a five hour old pupa. It showed with startling clarity a series of "goblet-like" structures arranged in a very regular manner, their large diameter directed away from the optic nerve (Fig. 4). Further investigation made it clear that these "goblets" were the result of longitudinal sections through structures that, in cross section, had the appearance of the cell "clusters" described above. This is well illustrated by Fig. 5, a photograph of a section through the eye disk of a six hour old pupa. A search for these structures was made in 72- and 96-hr. larvae. They were found in larvae of both stages—many more of them in 96-hr.- than in 72-hr.-old larvae. Fig. 2 is a photograph of the "goblets" as seen in a 96 hr. old wild type larva. That these "goblet-like" structures are the precursors of the ommatidia is clearly shown by a study of later and later pupal stages (Figs. 1 through 13). The "goblets" undergo various minor changes in shape during their development. Just after eversion of the eye disks they are somewhat foreshortened and "wedge-shaped" (Fig. 6). Six hours later they are again "goblet-shaped" (Fig. 8). After another six hours they are again foreshortened and "wedge-shaped", differing from the 12 hr. stage in coming to a sharper wedge (Fig. 9). At all these stages it is possible to trace in at least some regions a single nerve fibre entering each "goblet", in favourable locations these fibres can be seen separating from the

large, common optic nerve (Figs. 6 and 9). This has been observed in larval as well as pupal stages. During the next 24 hr. the ommatidial precursors become very much foreshortened, as though compressed, and assume in longitudinal section (i.e. longitudinal section through them) an almost circular shape. The nervous connection is much clearer (Fig. 10). In addition there is now, for the first time, clear indication of the rhabdome, which is very clearly seen in cross sections through the ommatidia (Fig. 11). The cross sections show in addition that another cell division has occurred, since they now show eight instead of four cells in each ommatidial precursor. In the next 24 hr. the ommatidia differentiate quite rapidly assuming very nearly their definitive form (Fig. 13), undergoing only further elongation in subsequent development. At this time, the description of the adult eye published by Johannsen (8) or Hertweck (6) is applicable without change except as to length and pigmentation.

Bar

Re-examination of the eye disks of Bar larvae showed that the "goblets" (the structures seen when the ommatidial precursors are cut longitudinally) were present in them just as in wild type. They made their first appearance at 72 hr. after hatching (Fig. 15) and as in the wild type increased in number between 72 and 96 hr. of larval life (Fig. 17). The number of "goblets" in the Bar eye disks is many fewer than that in the wild type. For example, at no time were more than four "goblets" seen in any one section of Bar eye disks while more than 20 were seen in a single section of a wild type eye disk. The low frequency with which the "goblets" (ommatidial precursors) are present in Bar accounts for the author's failure to detect the "clusters" (the structures seen when the ommatidial precursors are cut in cross section) in the Bar eye disks, while they were readily seen in the wild type (19). A renewed search for the "clusters" again resulted in failure, or at least uncertainty. This may be expected since a section through four ommatidial precursors would result in a group of four cell clusters, a figure that may easily be confused with a shrinkage artifact. It is precisely because of this difficulty that the author hesitates to say with certainy that the "clusters" have been seen in Bar eye disks, nevertheless, it is obvious that they must have been present in some preparations since it is unlikely that there were no cross sections through the ommatidial precursors. In their subsequent development the Bar "goblets" parallel the wild type in every detail (Figs. 18 and 19).

Special attention was paid to the pigmented, non-facetted region of the eye to check its mode of development. This region is of particular importance in the light of Wolsky and Huxley's (25) report that ". . . . . the (pigmented) non-facetted regions seem to be rather degenerate in their structure. . . . . the non-facetted area consists of distinct blocks of rather hypertrophied cells, while between the blocks some narrow space can be distinguished . . . . . these solid blocks of heavily pigmented cells correspond to rudimentary "ommatidia", in which, however, the elements of the ommatidia proper . . . . . are entirely lacking. Thus the blocks consist only of accessory



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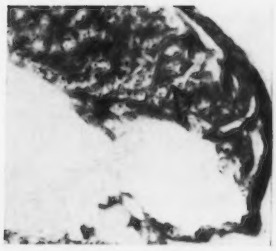

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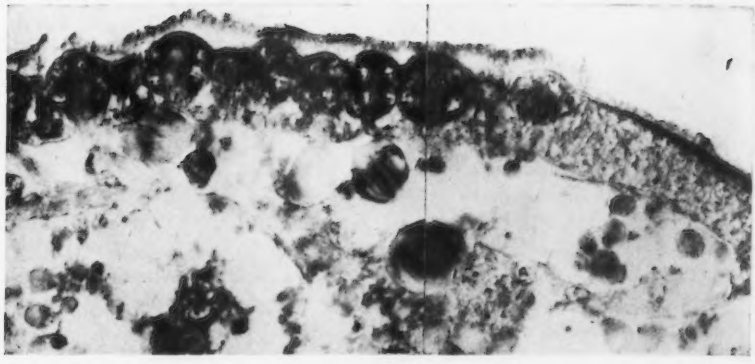

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All prints are contact prints. All magnifications given are initial magnifications at the ocular. B = brain; E = eye disk; L = limb bud.

FIG. 14. *Section through a Bar larva 72 hr. after hatching. 140×.*

FIG. 15. *High power photograph of a portion of the optic disk shown in Fig. 14. 840×. The arrow indicates a "goblet".*

FIG. 16. *Section through a Bar larva 96 hr. after hatching. 140×.*

FIG. 17. *High power photograph of a portion of the right optic disk shown in Fig. 16. 840×. The arrow indicates a "goblet".*

FIG. 18. *Cross section through the eye of a 24 hr. Bar pupa. 840×. The arrow indicates a "goblet" and its nerve.*

FIG. 19. *Cross section through the eye of a 48 hr. old Bar pupa. The future pigmented, unfacetted region is at the right. 840×.*

elements which normally form the pigmented coat around the retinula, but here take its entire place. . . . . in some cases there is no sharp demarcation between the structure of the facetted and non-facetted regions and one can find degenerated ommatidia which still contain some rudiments of the retinula and dioptric apparatus showing also rudimentary facet formation in the overlying cuticle." These observations lend support to those hypotheses requiring a secondary destruction of already formed material.

The pigmented, non-facetted region can easily be recognized at 48 hr. after puparium formation. At this time it appears as a compactly organized tissue with no evidence of organization into ommatidial precursors (Fig. 19). Many slides were examined and this point was checked and rechecked. At 72 hr. after puparium formation the only changes that the pigmented, non-facetted region has undergone are (*a*) a very heavy deposition of pigment and (*b*) an elongation. As at 48 hr., there is no evidence of degenerate ommatidia; *there are no ommatidia in this region at any time.* It seems quite likely that the "degenerate ommatidia" that Wolsky and Huxley believed they saw were simply oblique sections through a portion of some ommatidia that border the non-facetted region. The author has seen such sections in his own preparations. However, if several successive sections are studied, the nature of the structures becomes clear.

Although slides of 30-, 36-, and 42-hr. pupae were studied with the hope of obtaining an insight into the origin of the pigmented, non-facetted region, no satisfactory evidence as to the origin of this region was obtained. Undoubtedly further study would be rewarded with success but since this question is not pertinent to the problem under investigation it was not pursued further.

Some Observations on the Eversion of the Cephalic Complex

During these investigations some observations on the course of the evagination of the cephalic complex were made that may serve to supplement those reported by Robertson (18). During the prepupal period the position of the optic disk changes from its original one of close apposition to the brain to a position just within the anterior opening of the frontal sac (Fig. 7). This new position is attained by the gradual rotation of the disk anteriorly. It reaches its most advanced point at about nine hours after puparium formation. Sometime between 11½ and 12 hr. after puparium formation, the disks are suddenly everted (Robertson, 18 and the author). During these manoeuvers of the optic disk, the optic nerve follows faithfully along. This is clearly shown in Fig. 7. It seems clear therefore that the nervous connection established at least as early as 24 hr. after hatching from the egg (19) is maintained throughout the remainder of development. The situation described here is identical with that described by Van Rees (22) in *Musca vomitoria* and by Miall and Hammond (13) in *Chironomus*. As a matter of fact, the figures and descriptions of the metamorphosis of the head given in these papers may be applied unchanged to *Drosophila*.

What remains unclear is the origin of the nerve fibres connecting the ommatidia and the brain. The optic stalk is established long before the ommatidial precursors can be seen. It increases in thickness as development proceeds, i.e. more nerve fibres develop. Moreover there is a clear relationship between the number of ommatidia and the number of fibres that develop. This is most sharply brought out by the demonstration that the fewer the ommatidia the greater the reduction in the optic ganglia of the imago (8 and 17). The fact that there is no associated reduction in the larval dorsal ganglia (12 and 14) is not surprising since there are no true optic ganglia in the larval brain (6).

The nerve fibres may originate in the larval brain and then make connection with the ommatidia. Alternately the fibres may originate at the ommatidia and then grow toward the brain. There are no data available at present that would aid in deciding between these alternatives. The fact that in *Drosophila* the optic disk will develop normally when transplanted without any connection to the brain, may afford a method of deciding the question. It would be necessary to transplant disks from very young larvae (e.g. 24 hr. after hatching) and then study the innervation of the implanted eye after metamorphosis. Pilkington (14) by transplanting eye disks of mature larvae into similar hosts, obtained confirmation of Weismann's suggestion that the outer optic ganglion forms from the optic stalk. Transplantation at this stage, however, is much too late to study the origin of the nerves since they are already present and are to some extent organized into a ganglion.

Discussion

The data presented above show that the histological differentiation of the "clusters" and "goblets", depending on the plane of section, begins between 48 and 72 hr. after hatching at 25° C. in Bar just as in wild type; that thereafter the histological picture is the same in both forms with the exception of the pigmented but unfacetted region of the Bar eye. They show quite definitely that these structures (the "clusters" and "goblets") are the precursors of the ommatidia. Furthermore, the data establish that the pigmented, unfacetted region of the Bar eye does not result from a degeneration of already formed ommatidia.

These facts are further contradictions to those hypotheses that involve a destruction of already formed materials to explain the development of the Bar eye. The histological evidence taken in conjunction with the evidence derived from a study of the growth curves of the eye disks of Bar, modified Bar, and wild type (19) makes it clear that all such hypotheses as well as those involving different growth rates of Bar and wild type must be discarded. On the other hand, these data lend further support to the hypothesis advanced by the author (19 and 20) that the primary morphological effect of the Bar mutation is to reduce the capital of cells going into the formation of the cephalic complex; more specifically, into that portion of the cephalic complex that will eventually give rise to the eye disk. In its complete form the

hypothesis postulates, in addition, the presence of a group of cells labilely determined to form either facets or head chitin. The ultimate fate of these cells depends upon the nature of various extrinsic and intrinsic modifiers that may be present. It is the presence of these labilely determined cells that permits the variation of eye size observed within a given genotype. This hypothesis may suffice to explain the development of other mutants that reduce the facet number of the eye, e.g. eyeless, glass, lobed, etc., however, direct tests are desirable and these tests are under way.

One phase of the problem of the development of the Bar eye remains unexplored, namely, the question of the shape of the eye and the distribution of the facets within it. The present studies shed no light on the origin of the pattern of the Bar eye. It must be emphasized, however, that a complete explanation of the mode of development of the Bar eye will not be available until this phase of the problem is understood. This is equally true of all other mutations that affect facet number.

The author recognizes that his hypothesis falls short in that it does not explain the origin of the Bar pattern. Nevertheless, it is necessary to explain the method of facet number reduction as a preliminary to an explanation of the pattern woven out of this reduced number of facets. Studies such as the one reported above are directed toward that end.

References

1. AUERBACH, C. Trans. Roy. Soc. Edinburgh, 58 : 787-815. 1936.
2. BODENSTEIN, D. Arch. Entwicklungsmech. Organ. 137 : 474-505. 1938.
3. CHEN, T. Y. J. Morphol. Physiol. 47 : 135-199. 1929.
4. ENZMANN, E. V. and HASKINS, C. P. J. Morphol. 63 : 63-73. 1938.
5. GOLDSCHMIDT, R. Univ. Calif. Pub. Zool. 41 : 277-282. 1937.
6. HERTWECK, H. Z. wiss. Zool. 139 : 559-663. 1931.
7. HUXLEY, J. S. Biol. Zhur. 4 : 421-425. 1935.
8. JOHANNSEN, O. A. J. Morphol. Physiol. 39 : 337-349. 1924.
9. KALISS, N. Genetics, 24 : 244-270. 1939.
10. KRAFKA, J., JR. Biol. Bull. 47 : 143-148. 1924.
11. MARGOLIS, O. S. and ROBERTSON, C. W. Genetics, 22 : 319-331. 1937.
12. MEDVEDEV, N. N. Z. ind. Abst. Vererb. 70 : 55-72. 1935.
13. MIALL, L. C. and HAMMOND, A. R. Trans. Linnean Soc. Zool. (Ser. 2), 5 : 265-280. 1892.
14. PILKINGTON, R. W. Proc. Zool. Soc. London (Ser. A), 111 : 199-222. 1941.
15. POULSON, D. F. Actualités sci. 498 : 1-51. 1937.
16. POULSON, D. F. J. Exptl. Zool. 83 : 271-325. 1940.
17. RICHARDS, M. H. and FURROW, E. Y. Biol. Bull. 48 : 243-258. 1925.
18. ROBERTSON, C. W. J. Morphol. 59 : 351-399. 1936.
19. STEINBERG, A. G. Genetics, 26 : 325-346. 1941.
20. STEINBERG, A. G. Genetics, 26 : 440-451. 1941.
21. STEINBERG, A. G. Genetics, 27 : 171-172. 1942.
22. VAN REES, J. Zool. Jahrb. 3 : 1-134. 1889.
23. WADDINGTON, C. H. J. Genetics, 41 : 75-139. 1940.
24. WEISMANN, A. Z. wiss. Zool. 14 : 187-336. 1864.
25. WOLSKY, A. and HUXLEY, J. S. Proc. Zool. Soc. London, Pt. II : 485-489. 1936.

STUDIES ON CESTODES OF THE GENUS *TRIAENOPHORUS* FROM FISH OF LESSER SLAVE LAKE, ALBERTA

II. THE EGGS, CORACIDIA, AND LIFE IN THE FIRST INTERMEDIATE HOST OF *TRIAENOPHORUS CRASSUS* FOREL AND *T. NODULOSUS* (PALLAS)[1]

By Richard B. Miller[2]

Abstract

The eggs of *Triaenophorus crassus* vary from 53 to 68 μ long by 38 to 44 μ in diameter. Those of *T. nodulosus* are practically the same ranging from 58 to 67 μ long by 38 to 44 μ in diameter. Movement of the embryo within the egg is discernible two days before hatching. The eggs of one individual hatch over a period of eight to 10 days.

The coracidia of *T. crassus* are from 67 to 80 μ long by 49 to 58 μ wide and of *T. nodulosus* from 67 to 85 μ long by 58 to 80 μ in diameter. The coracidia live for two or three days and are free swimming for most of this time.

The first intermediate host of both species in Lesser Slave Lake is *Cyclops bicuspidatus* Claus; the coracidia of both species will also infect *Diaptomus ashlandi* Marsh, but fail to develop in this copepod. The procercoids develop in the body cavity of the *Cyclops* in eight to 10 days after their entry; both species attain in this time an average length of 300 μ. Where a large number of procercoids occurs simultaneously in one *Cyclops* the growth rate is slower and the maximum size attained is less.

The Eggs and Hatching

In Part I of these studies (4) the maturation of *T. crassus* and *T. nodulosus* was described, and the times and manner of egg-laying of each detailed. For the former the egg-laying period is the first half of May; for the latter it is mostly in early June, about one month later.

The eggs of both species are white in colour but turn brown about 45 min. after being placed in water. They are "egg-shaped" and nearly the same size; in *T. crassus* they vary from 53 to 68 μ long by 38 to 44 μ in diameter (average 61 × 41 μ) and in *T. nodulosus* from 58 to 67 μ long by 38 to 44 μ in diameter (average 61 × 44 μ).

In the ripe eggs the onchospheres can be clearly made out (Fig. 1). Two days before eclosion faint movements of the embryo are discernible. These become more and more violent and culminate in the popping open of the operculum at the small end of the egg and the emergence of the coracidium. The round aperture revealed when the operculum opens is much smaller in diameter than the coracidium which is forced to struggle considerably to pass through it; hatching usually lasts from 15 to 30 sec. Several times coracidia were seen that had failed to get entirely out of the egg and were swimming around dragging it with them. Sometimes the operculum bursts completely off the egg during hatching but in most eggs it remains attached by one side (Fig. 2).

[1] *Manuscript received June 11, 1943.*
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PLATE I

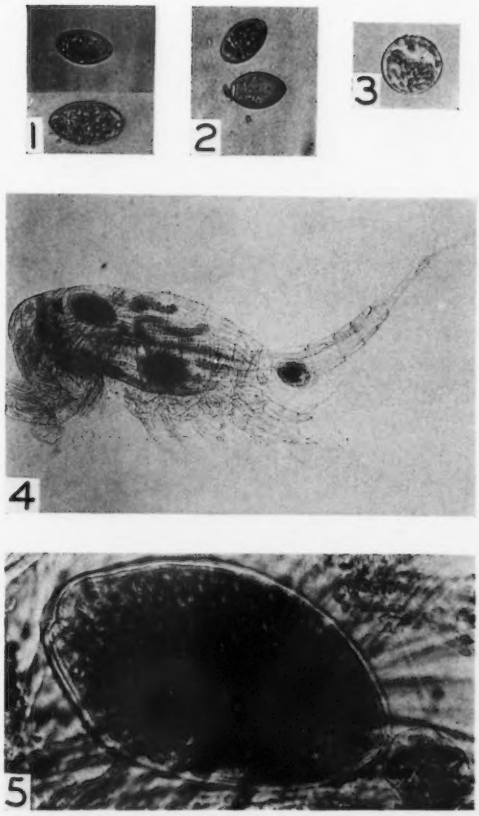


FIG. 1. *Photomicrographs of two living eggs of Triaenophorus crassus just prior to hatching; the outlines of the coracidia can be seen through the transparent egg shells. Upper egg magnified 148 times, lower 189 times.*

FIG. 2. *Photomicrograph of the empty egg of Triaenophorus crassus; the operculum and the hole through which the coracidium escaped can be seen. Shown also is an unripe, probably dead, egg.* ×*148.*

FIG. 3. *Photomicrograph of the living coracidium of T. crassus about two hours old.* ×*189.*

FIG. 4. *Photomicrograph of a stained whole mount of Cyclops bicuspidatus containing two mature procercoids of Triaenophorus crassus.* ×*60.*

FIG. 5. *The anterior procercoid of the two shown in Fig. 4 photographed through an oil immersion lens; the cercomere and two of its hooks and the invagination in the future scolex region can be distinguished.* ×*390.*

Detailed observations were made on the hatching of the eggs from over a dozen different specimens of *T. crassus*. The eggs from each worm were placed in a Petri dish and covered with approximately 1 cm. of water. Every day at about the same hour the eggs were stirred and a sample pipetted to a glass slide and examined microscopically. With the aid of a Whipple disk, counts of the relative numbers of hatched and dead eggs were made. At the same time the numbers of living and dead coracidia were estimated. The results of some of these observations are shown in Table I.

TABLE I

THE DURATION OF HATCHING AND LENGTH OF LIFE OF THE CORACIDIA AS OBSERVED IN FIVE CULTURES OF EGGS OF *T. crassus*

Days after incidence of hatching	0	1	2	3	4	5	6	7	8	9	10
Culture 1											
Eggs hatched, %	0.1	4	30	60	52	81	84	86	—	—	94
Eggs dead, %	—	—	—	—	—	—	17	6	—	—	6
Coracidia dead, %	—	—	—	—	—	—	—	—	—	100	100
Culture 2											
Eggs hatched, %	1.7	12	16	14	48	41	59	48	56	50	—
Eggs dead, %	—	—	—	—	—	24	29	40	43	50	—
Coracidia dead, %	—	0	16.7	25	20	12	33	76	88	100	—
Culture 3											
Eggs hatched, %	9	12	37	54	61	70	—	—	75	—	—
Eggs dead, %	—	—	—	20	30	28	—	—	25	—	—
Coracidia dead, %	—	0.2	41	27	41	70	60	—	75	—	100
Culture 4											
Eggs hatched, %	0.1	—	78	—	74	—	86	—	98	—	—
Eggs dead, %	—	—	—	—	—	—	—	—	—	—	—
Coracidia dead, %	—	—	38	—	19	—	56	—	100	—	100
Culture 5											
Eggs hatched, %	0.1	2.9	5.8	11.5	42.7	50	50	74	55	61	74
Eggs dead, %	—	—	—	—	—	26	35	25	43	38	25
Coracidia dead, %	—	—	—	—	—	—	69	85	97	96	100

The temperature in the room where the cultures of eggs were kept varied from 55° to 70° F.; most of the time it remained in the fifties.

Hatching continued for as long as 10 days after it began; in some cultures half the eggs had hatched by the third day and usually most of the viable eggs had hatched by the end of a week. Cultures of the eggs of *T. nodulosus* were much the same; hatching usually continued for eight days and occasionally for 10.

Noteworthy is the high viability of some of the cultures of *T. crassus* eggs; a fully ripe adult of *T. crassus* was estimated to contain 1,175,000 eggs; in one culture 94% of all these eggs hatched; in another, believed to be exceptional, only 50% of the eggs proved to be viable. Many cultures for which

no accurate records were kept appeared to have very few dead eggs when hatching was completed.

The data, here reported, on the dimensions of the eggs of *T. crassus* and the duration of hatching are in agreement with similar data published by Ekbaum (1) for her Canadian material.

The Coracidia

Immediately after eclosion the coracidia are about the same size as the egg, sometimes smaller. For about an hour they swim so rapidly that it is impossible to catch more than fleeting glimpses of them in the field of a compound microscope. During this period they gradually increase in size. This increase is due to the swelling of the single layer of ciliated cells with which each is invested; the actual onchosphere remains the same size as it was in the egg just prior to hatching. When their full proportions are attained their movements are quite slow and they can be readily observed. They are shaped rather like a hen's egg and swim with the broader, hook-free end anterior. During forward movement they rotate slowly first in one direction, then in the other, alternating irregularly. The cilia responsible for this movement can be made out with the high powers of a microscope, especially with the aid of a dark field condenser. The present study was made in the field and proper facilities for such work were not available; Dr. Ekbaum's measurements of the cilia of the coracidia of *T. crassus* are quoted here. She states these to be 10 to 15 μ long with a special tuft 30 to 35 μ long at the end directed forward when the coracidia are swimming (1). Observations the author was able to make are in agreement with these findings.

Coracidia of *T. crassus*, one day old, ranged in size from 67 to 80 μ long by 49 to 58 μ wide (average 73 $\times$ 54 μ) (Figs. 3 and 6). Ekbaum (1) found

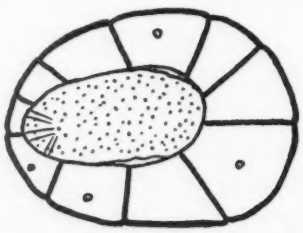


FIG. 6. *Camera lucida drawing of a two day old coracidium of Triaenophorus crassus. The cilia are not shown.* $\times 600$.

the coracidia of *T. crassus* to measure 60 to 70 μ in length and 50 to 60 μ in diameter. Newton (5) stated that the coracidia of *T. tricuspidatus* (presumably *T. crassus*) "are extremely small, measuring six μ in diameter". Furthermore he states that the coracidia appeared 15 days after placing the eggs in water; in the experience of the present investigator the eggs would be dead after this lapse of time. Newton's coracidia also failed to infect various

species of copepods that were exposed to them; he states that, ". . . . . the coracidia remained alive for over a month . . . . .". This is a much longer coracidial life span than has been found by other workers and in the present study. In view of these discrepancies it is possible that Newton measured organisms of some kind that developed in his culture and that were not coracidia.

Coracidia of *T. nodulosus* are of nearly the same proportions as those of *T. crassus*; they tend to be somewhat broader, ranging from 67 to 85 μ long by 58 to 80 μ wide (average 75 $\times$ 68 μ).

In Table I the percentage of dead coracidia found each day in some of the egg cultures of *T. crassus* is shown. A few coracidia appear to live for only 24 hr. Most of them probably live for at least twice this time. During most of its life the coracidium is free swimming; before dying it settles on the bottom. A few coracidia were observed to use their hooks to work their way out of their ciliated coats; they then crept around slowly on the bottom of the culture dish for a short time. The majority, after settling, died within their ciliated investment. These observations apply particularly to *T. crassus* but *T. nodulosus* is the same in every respect.

Ekbaum (1) states that "after three to four days the coracidium gradually loses its cilia, ceases the movements and remains on the bottom of the dish. In several cases it was noted that the outer covering with the cilia was cast off." These observations on *T. crassus* are essentially the same as those reported here.

The power of infecting the first intermediate host was tested at different times during the period that a culture still possessed live, unhatched eggs and living coracidia. During the week following the incidence of hatching the egg and coracidia cultures of *T. crassus* were capable of infecting close to 100% of the first intermediate hosts that were exposed to them. The latest successful infections were obtained with cultures eight days after hatching began; in this experiment only two out of 22 *Cyclops* became infected; living onchospheres were found in pellets defecated by some of the *Cyclops* in these cultures; they had evidently been swallowed by the *Cyclops* but lacked the strength to burrow through the gut wall into the body cavity.

Development of the Procercoid in the First Intermediate Host

The field laboratory set up for this study was on the shore of Lesser Slave Lake; plankton was secured from the lake as the need arose and inoculated with egg and coracidia cultures of *T. crassus* and *T. nodulosus*. The two most abundant plankters in the shallow waters were the copepods *Cyclops bicuspidatus* Claus and *Diaptomus ashlandi* Marsh. Both of these became infected with either *T. crassus* or *T. nodulosus*, but *Cyclops bicuspidatus* is the natural host; in none of the experiments was a procercoid of either species observed to reach maturity or grow to any appreciable extent in *Diaptomus*. Two other copepods, *Cyclops albidus* Jurine and *C. viridis* Jurine were also

tried as possible hosts. No infections of *C. albidus* with either *T. crassus* or *T. nodulosus* were observed and only one *C. viridis* was found that contained a parasite (young procercoid of *T. nodulosus*). Various cladocerans including *Daphnia pulex* (de Geer) and *D. longispina* (O. F. Müller) proved immune to infection by either species of parasite.

Michajlow (3) made a detailed study of the ability of coracidia of *T. nodulosus* to infect different species of copepods. (This paper is known to the author only through an abstract.) He found five types of copepod as follows:—

1. The digestive juice of the copepod killed the coracidia in the gut: *Diaptomus amblyodon* Maren, *D. castor* Jurine, and *Cyclops viridis* Jurine.

2. Most of the parasites killed by digestive juice but a few get into the body cavity: *Cyclops orthonoides* Sar and *C. leuckarti* Claus.

3. Majority of the coracidia enter the body cavity but fail to develop: *Cyclops serrulatus* Fischer.

4. A few parasites get into the body cavity and develop normally: *Cyclops albidus* Jurine, *C. insignis* Claus, *C. fuscus* Jurine, *C. vernalis* Fischer, *C. bicuspidatus* Claus, and *Diaptomus gracilis* Sar.

5. All coracidia get from gut to body cavity and develop normally: *Cyclops strenuus* Fischer.

Cyclops strenuus, the natural host in Europe, was not found by the author in any of the Alberta lakes investigated (14 lakes). *Cyclops bicuspidatus*, however, was abundant in all of them. Usually all the coracidia swallowed by this copepod successfully negotiated the gut wall but occasionally some were seen to be defecated. The coracidia of both *T. crassus* and *T. nodulosus* were most successful in establishing themselves in the nauplii and young adults. *Diaptomus ashlandi* would seem to belong in Michajlow's third group according to the observations recorded here.

The study of the development of the procercoids of both *T. crassus* and *T. nodulosus* was made using *Cyclops bicuspidatus* as the host. Within one hour of the time of adding coracidia of *T. crassus* to a culture of these copepods the parasites could be found in their stomachs and body cavities; each *Cyclops* usually contained four or five by this time; of these one or two were in the body cavity and the remainder were still in the stomach where they could be observed digging through the stomach wall. All the parasites had lost their ciliated coats. The interesting process of penetrating the stomach wall was observed in some detail; each parasite swings its six hooks backward in such a manner that the ends come together to form one pointed tool; this point is then thrust into the stomach wall a short distance; the hooks are then slowly and forcefully spread apart so as to enlarge the breach in the wall. This process is repeated several times until a large enough hole is torn in the stomach for the parasite to wriggle through into the perihaemal space of the *Cyclops*. At each thrust of the hooks the stomach muscles of the *Cyclops* contract violently; often the animal's whole body gives obvious signs of

distress by spasmodic jerkings, particularly of the abdomen and antennae. On one occasion the contractions of the *Cyclops'* stomach were so violent that all the parasites contained in it were forcibly ejected through the mouth and back into the water, where they crawled about for a short time and then died.

The use of the coracidial hooks described in the preceding paragraph appears to be the only one; the hooks were often moved afterward, but were never put to any apparent use.

The subsequent growth of the parasite from the coracidium to the mature procercoid was observed in many hundreds of *Cyclops* for both *T. crassus* and *T. nodulosus*. More careful records of these observations were kept for *T. crassus* and a summary of them for three cultures, two lightly and one heavily infected, is shown in Table II. While these observations apply particularly to *T. crassus*, the facts are practically the same for the other species.

TABLE II

THE GROWTH OF *Triaenophorus crassus* FROM THE CORACIDIUM TO THE MATURE PROCERCOID IN THE BODY CAVITY OF *Cyclops bicuspidatus*[1]

	Age of procercoid, days								
	1	2	3	4	5	6	7	8	9
	Length, μ								
Culture 1[3]	—	62	—	—	—	155	—	310[2]	—
Culture 2[4]	50	80	—	110	—	220	—	352[2]	—
Culture 3[5]	—	80	75	80	—	160	175	89	220
	Age of procercoid, days								
	10	11	12	13	14	15	16	17	
	Length, μ								
Culture 1	—	220[2]	—	—	—	—	—	185[2]	
Culture 2	330[2]	—	310[2]	—	—	265[2]	—	—	
Culture 3	200[2]	—	—	—	200[2]	—	—	—	

[1] *The figures in the table are the lengths in μ of the largest procercoids.*
[2] *Indicates that a cercomere was developed.*
[3] *Culture 1, average of 2.7 procercoids per Cyclops.*
[4] *Culture 2, average of 2.8 procercoids per Cyclops.*
[5] *Culture 3, average of 10 procercoids per Cyclops.*

When the coracidium first arrives in the body cavity of the *Cyclops* it is the same size as it was as a free living organism within its ciliated investment. After two days in the *Cyclops* a small increase in size can be noticed. Subsequent growth is rapid; by four days its size is doubled and by eight days it has reached its mature size of 300 to 350 μ, roughly six times the length of the coracidium. The procercoid was taken as mature when the cercomere, or caudal appendage, was clearly pinched off and the terminal invagination (frontal gland) in the future scolex region could be distinguished (Figs. 4

and 5). Also visible was the complex excretory system leading to an invagination at the posterior end where the stalk of the cercomere is attached; eight symmetrically disposed calcareous particles, four in an anteroposterior line on each side, were usually discernible as well. The length of the procercoid at this stage varied from 55 to 352 μ, but was usually close to 300 μ. The cercomere varied from 25 to 50 μ in length.

After the cercomere is established the procercoid either stops growing or grows very slowly. Cultures of copepods were kept alive for as long as 22 days after infection with coracidia of *T. crassus*; no increase of size of the procercoids was observed after the eighth day.

The number of parasites per host has some effect on the growth rate. This number ranged from 1 to 32 in a single *Cyclops*. Two of the cultures recorded in Table II averaged 2.7 and 2.8 procercoids per *Cyclops*; procercoids in these reached their maximum size in eight days. In the third culture in Table II the average number of developing procercoids per *Cyclops* was 10. In this culture the growth of the procercoids was little affected until after the fourth day; the overcrowding factor then began to operate; mature procercoids were not found until the 10th day, two days later than in the less heavily infected cultures; the largest found measured only 200 μ, about two-thirds the size attained in the other cultures.

During the whole of their lives within the *Cyclops* the procercoids display active movement; they are never attached to any part of the host's body. They constantly lengthen and shorten, gliding from one part of the host to another. Young procercoids were frequently found in the antennae and caudal rami as well as the more usual locations in the thorax and abdomen. Their presence seemed not to affect the *Cyclops* appreciably. As mentioned above parasitized *Cyclops* lived for a period of 22 days and might have lived longer had they been free from the rough handling involved in microscopic examination. Janicki (2) noted that *Cyclops* heavily infected with procercoids of *T. nodulosus* tended to settle to the bottom of their culture dish and become covered with epiphytes. This was noted in the present investigation for *Cyclops* heavily parasitized with either *T. crassus* or *T. nodulosus*.

Procercoids of either species have never been observed to lose their cercomeres while still in the body of the host. This appendage is very lightly attached, however, as a slight pressure on the coverslip during microscopic examination will cause it to drop off.

The number of procercoids of *T. nodulosus* per *Cyclops* was observed to vary from 1 to 27. The development of the procercoid is essentially the same as described above for *T. crassus*. Janicki (2) found in his studies of *T. nodulosus* in *Cyclops strenuus* that the procercoid stage was reached in 11 days. The maximum size of the procercoids in his investigation was 714 μ attained at the end of a month. In the present study procercoids of *T. nodulosus* reached only a little over half this size in about 20 days. The fact, mentioned above, that Michajlow has shown that *Cyclops bicuspidatus* is not the natural host for this species may explain this difference in the size of the procercoid.

Acknowledgments

In addition to the help received from the Fisheries Branch of the Government of the Province of Alberta, acknowledged in Part I of these studies, the author would like to express his gratitude to Mr. J. E. Moore, formerly of the Department of Biology, University of Toronto, now of the R.C.A.F. Mr. Moore kindly checked the author's identification of the copepods from Lesser Slave Lake.

References

1. EKBAUM, E. J. Parasitol. 23 : 293-295. 1937.
2. JANICKI, C. Korresp.-Bl. Schweiz. Aerzte, 48 : 1343-1349. 1918.
3. MICHAJLOW, W. Ann. parasitol. humaine comp. 10 : 334-344. 1932.
4. MILLER, R. B. Can. J. Research, D, 21 : 160-170. 1943.
5. NEWTON, M. V. B. Contrib. Can. Biol. Fisheries (n.s.), 7 (28) : 341-360. 1932.

VARIATION IN THE PORCUPINE (GENUS *ERETHIZON*) IN CANADA[1]

By R. M. Anderson[2] and A. L. Rand[3]

Abstract

The porcupines of Canada (genus *Erethizon*) have usually been referred to two distinct species. A survey of material in the National Museum of Canada shows that they actually represent but one species, *Erethizon dorsatum*, with five geographical races, which are summarized in this paper. Each form is differentiated by a mosaic of external and internal characters, some of which frequently occur in other forms. There is considerable individual variation. Some overlapping of characters through individual variation occurs in widely separated populations. Where two rather distinct subspecies meet, intergrades occur, but intergradations in skull characters do not always occur in the same geographical area as intergradations of pelage characters.

Introduction

The porcupines of Canada present some interesting aspects of variation in both pelage and skull characters. Hollister (9, p. 27), considering specimens from Jasper National Park, says that the skulls of this porcupine exhibit more individual variation than do those of any other mammal that he has carefully studied. However, individual variation appears to be greater in some areas than in others, as he says Alaska specimens are remarkably uniform throughout a large series. Swarth (15, pp. 380–381) discussing Skeena River specimens mentions variability in colour, but suggests that cranial characters vary geographically. Bangs (3, pp. 37–39) mentions the variability in both skin and skulls of eastern porcupines, but considered it in part correlated with geography. Allen (1, p. 391) has pointed out the variation in skulls from Maine.

Our material shows that while in some populations there is great variation, as in the pelage of Teslin Lake specimens and in the skulls from southern British Columbia, in others there is much less variation, as in the pelage of skins from Eastern Canada, and in skulls from the Bella Coola area of British Columbia, and that some variation is geographical.

Two species are currently recognized in the genus *Erethizon*, both occurring in Canada, a black-haired eastern porcupine, *E. dorsatum* Linnaeus (10, p. 57), with two subspecies, and a yellow-haired western porcupine, *E. epixanthum* Brandt (5, p. 390), with several subspecies.

The yellow-haired porcupine has been considered as ranging over the plains and mountains from Turtle Mountains, N.D. and Wood Buffalo Park westward, and the black-haired porcupine, mainly to the east of that but occasionally occurring as far west as northern British Columbia, its range thus overlapping that of the yellow-haired form.

[1] *Manuscript received June 11, 1943.*
Contribution from the Division of Biology, National Museum of Canada, Ottawa, Canada.
[2] *Chief, Division of Biology, National Museum of Canada.*
[3] *Assistant Zoologist, National Museum of Canada.*

Porcupines are awkward things to handle, and this has retarded building up of series of specimens and their study. In the National Museum of Canada we have a series of some 63 skins and 71 skulls from many parts of Canada. There still are areas from which we need specimens to determine the limits of forms and the status of populations. Especially do we need material from the southern part of the Prairie Provinces, from the area between northern Manitoba and the Yukon, and from Ungava. But a survey of the material available brings out some interesting points.

It appears that the yellow-haired and the black-haired porcupines intergrade; in pelage notably in the southern Yukon, in the vicinity of Teslin Lake, and in skull characters in the Wood Buffalo Park area at least. This makes it necessary to consider the porcupines of the genus *Erethizon* as belonging to one species instead of two, and the names of the forms (see Fig. 1) occurring in Canada should stand as follows:

A. Erethizon dorsatum picinum Bangs (1900)
B. Erethizon dorsatum dorsatum (Linnaeus) (1758)
C. Erethizon dorsatum myops Merriam (1900)
D. Erethizon dorsatum nigrescens Allen (1903)
E. Erethizon dorsatum epixanthum Brandt (1835)

Two additional forms have been described as subspecies of *Erethizon epixanthum* and are admitted as such to the North American list by Miller (13, p. 437), namely:

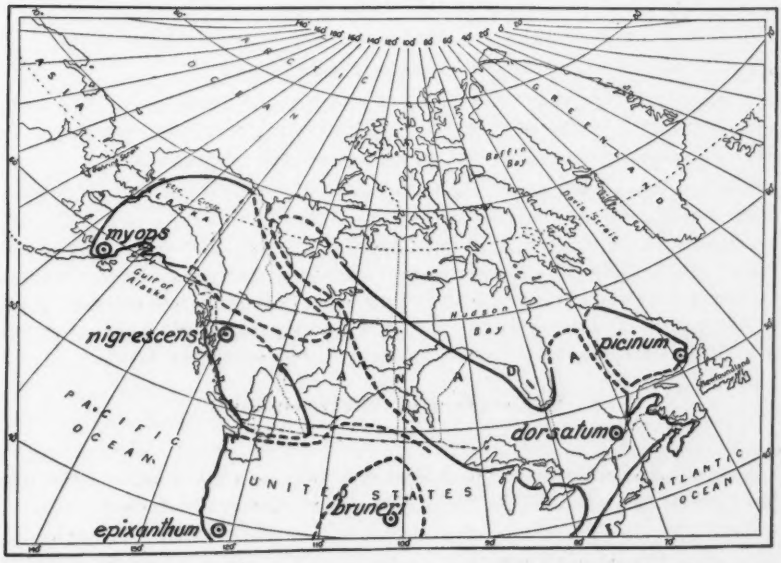


FIG. 1. *Geographic distribution of the races of Canada porcupine (Erethizon dorsatum) in Canada.*

1916. *Erethizon epixanthum bruneri* Swenk (17, pp. 115–125). Type locality, three miles east of Mitchell, Scottsbluff County, Neb. (near the Wyoming boundary). Nebraska porcupine.

1897. *Erethizon epixanthum couesi* Mearns (11, p. 723). Type locality, Fort Whipple, Yavapai County, Ariz. Arizona porcupine.

Both *bruneri* and *couesi* are described as paler and duller than the typical *E. epixanthum* of California, *bruneri* being slightly larger and *couesi* slightly smaller than *epixanthum*. The subspecies *bruneri*, if considered a tenable form, may be regarded as of doubtful occurrence in Canada, and *couesi*, the most southern race of the genus, is extralimital to Canada.

Allen (1, pp. 388–389) considered the two forms that he recognized, *dorsatum* and *epixanthum*, as varieties of one species and showed that they intergraded. Later, when he described *nigrescens* (2, p. 558) he considered it a race of the separate species *epixanthum*.

From a survey of our material the more important features that vary geographically were found to be as follows.

1. The general tone of the dark pigmented fur, from brownish to blackish.
2. The abundance of long guard hairs.
3. The abundance of yellowish or whitish tips to the guard hairs, and the width of these light tips.
4. The extent of whitish coloration on the head.
5. The shade of the whitish or yellowish coloration.
6. The extent and type of ridging on the cranium in the adult skulls.
7. The amount of swelling in the frontal bones.
8. The amount of depression in the frontal-parietal area.
9. The length of the nasals, and the shape of their posterior margin.
10. The development of a ridge on the sides of the premaxilla.

While all these characters vary geographically, they do not form an even trend in variation from one end of the population to the other; that is they do not represent a cline; intergradation appears to occur on a small part of the range only. There is also pronounced individual variation, so that individuals from widely separated populations often approach each other in some characters. Before going on to diagnose the recognizable forms in detail it may be advisable to discuss the variation in our series as a whole.

Explanation of Measurements

External Measurements

External measurements are in millimetres and are those made by the collector from the animal in the flesh. The following have been used:

Total Length.—The distance in a *straight line* from the tip of the nose to the end of the last tail vertebrae, exclusive of the hairs; animal lying on its back with the body stretched and the limbs pulled into a natural position.

Tail Vertebrae.—The distance from base of tail to tip of the terminal vertebra, exclusive of the hairs.

Hind Foot.—The distance from the end of heel bone (calcaneum) to the end of claw on the longest toe. The toes should be straightened out and this may be done by pressing them flat against the ruler, or the foot may be pressed flat on the table and the length measured with dividers.

Skull Measurements

Skull measurements are in millimetres and are made with caliper or dividers from clean, dry skull. The following have been selected as the most useful for making comparisons of specimens in this particular species:

Condylobasal Length.—Distance from the posterior border of the occipital condyle to the posterior edge of the alveolus of the incisor on the corresponding side of the skull.

Zygomatic Breadth.—Distance between the zygomatic arches at their widest point.

Length of Nasals.—From the most anterior point to most posterior point.

Width of Rostrum.—Greatest breadth of rostrum measured across anterior borders of the anteorbital foramina.

Age of course changes the appearance of skulls tremendously, so that only adult skulls are compared. Female skulls usually have the characters less pronounced, and must be compared separately. However we have few female skulls suitable for study. There is little difference in pelage between the sexes; in size females average smaller, but for external measurements it must be considered that porcupines are very difficult to handle and measure, and a high variability in measurements is to be expected; seasonal variation chiefly affects the under fur; it is long and completely conceals the spines in winter, short and scanty, leaving the spines mostly exposed in summer.

Albinos are not included in the present discussion. They are of occasional occurrence probably throughout the range of the species. We have specimens as follows:

Yukon: Whitehorse; partial albino, spines and fur white, except for broad rusty tips to guard hairs, nails whitish.

Alberta: Knee Hill Valley; partial albino, spines and fur white except for pale rusty tips to guard hairs, nails dusky.

Ontario: Ottawa; partial albino, white with a slight grey tinge in the pelage of dorsum, claws whitish.

Quebec: Near Chimo, south of Ungava Bay, a nearly pure white albino skin, without skull, was taken by natives and presented to the National Museum by Hudson's Bay Company through Mr. John Carroll of the Bureau of Geology and Topography in June, 1943.

We also have unpublished manuscript notes of a white porcupine from New Brunswick and two from western Quebec.

Schultz (14) recorded that in 53 porcupine skulls examined by him, 27 had bregmatic fontanelle bones present. In our series 62 skulls have the top of the skull complete, and only 10 of these show such extra bones. It should be pointed out that about half of the skulls in our series have the sutures obliterated, and, when younger, some may have had such accessory bones evident. Of our 10 specimens with bregmatic fontanelle bones, three had one; five had two; one had nine small ones in a cluster, and one had 10 small ones in a cluster.

TABLE I

DISTRIBUTION OF THE THREE COLOUR CHARACTERS OF THE PORCUPINE IN CANADA[1]

Locality	General ground colour[2]				Tint of yellow[3]					Extent of yellow[4]				
	Bl.	Br.Bl.	Bl.Br.	Br.	w.y.	p.y.	r.y.	o.y.	g.y.	A	B	C	D	E
Nova Scotia, New Brunswick, Gaspé (Quebec)	12				10	2				6	5	1		
Western Quebec, Ontario	8				8					4	3	1		
Manitoba		2			1	1					1	1		
Wood Buffalo Park, Alberta		1					1						1	
British Columbia and Jasper Park (Alberta)	14	1			1	4	2	8		1	6	6	3	
Yukon and Alaska	2	4	5	7		6	13			3	3	4	3	8
Cypress Hills (Alberta and Saskatchewan)			2	3	1	1			3		1	1	3	

[1] *The numbers represent the number of individuals, from the locality indicated at the left, with the character indicated at the top of the column.*

[2] *General tone of the under fur and guard hairs where not yellowish; Bl. = blackish, Br. Bl. = brownish black, Bl. Br. = blackish brown, Br. = brownish.*

[3] *Shade of yellowish tips to the guard hairs; w.y. = whitish, p.y. = pale yellow, r.y. = rusty yellow, o.y. = orange yellow, g.y. = greenish yellow.*

[4] *Amount of yellowish tipping; A: yellowish tips confined to nape, hips, and tail; B: yellowish tips also sparingly scattered over back; C: yellowish tips fairly well scattered over back but narrow; D: yellowish tips abundantly scattered over back but narrow; E: yellowish tips abundantly scattered over back and wide.*

A Survey of Variation (See Table I)

Pelage

From Ontario eastward porcupines are brownish black to black with pale yellow to yellowish-white tipping to the guard hairs; this is usually confined to nape, hips, and tail but in occasional specimens is sparingly scattered over the back.

Two northwest Manitoba specimens are more brownish with yellowish-white to yellowish guard hairs more or less sparingly scattered over the back.

A Wood Buffalo Park specimen is blackish, with abundant guard hairs, plentifully tipped with yellow.

A southern Yukon and Chitina River (Alaska) series is more variable; nearly half are brownish with profuse yellow guard hairs, giving a brownish yellow appearing animal; one-quarter are brownish black with few or no

yellowish-tipped guard hairs on the back and are barely distinguishable from eastern animals; one-quarter are intermediate between the above conditions.

British Columbia specimens and one Jasper Park specimen are brownish black to black, with yellow to orange-yellow tips to the abundant guard hairs; these tips are narrow to broad and scattered very sparingly to profusely over the back, giving the animal a black appearance, with yellow-tipped guard hairs; those with most yellow are not unlike intermediate Yukon specimens; those with least yellow approach one Nova Scotia specimen, but are more orange yellow.

The Great Plains specimens (southern Alberta and Saskatchewan) show considerable variation; three are similar to the yellow-phased Yukon specimens, but somewhat more greenish-yellow; one is like the intermediate Yukon specimens, and one is similar to the Manitoba specimens.

Skull Characters

The skulls of females show smaller ridges and crests, and differences appear in a lesser degree than in those of males. From Ontario eastward the skulls of adult males have a median swelling in the frontals and a slight depression in the frontal parietal area; cranial ridges are rather evenly developed throughout their length, sagittal and occipital crests moderate; nasals are short, with broadly rounded or subtruncate posterior margin; the cranial ridges and sagittal crests form a *Y* with long, straight arms; a small lateral ridge runs diagonally down across the side of the premaxilla, from the base of the rostrum (Figs. 3*a*, 3*b*).

A single, incomplete Manitoba skull (female) has very short nasals, with broadly rounded posterior tips and has the ridge on the side of the rostrum.

A Wood Buffalo Park adult female exhibits some of the characters of eastern Canada skulls (though the skin is of a "yellow-haired porcupine") and some of those of the Yukon type.

Yukon Territory and Chitina River, Alaska, adult males have longer nasals (on the average) with more pointed posterior margins; cranial ridge unevenly developed, with more development over the orbit; less frontal swelling and more of a frontal-parietal depression and a slight tendency toward a shortening and an inward bowing of the arms of the *Y* formed by the cranial ridges and sagittal crest; crests moderate; no ridge on the side of the rostrum (Figs. 4*a*, 4*b*).

British Columbia specimens (especially from the north) have no frontal swelling; very deep frontal-parietal depression; cranial ridges not evenly developed, but exaggerated over the orbit; crests greatly developed; arms of the *Y* formed by the sagittal crest and cranial ridges short, and much bowed inward; nasals long, with the posterior margin more pointed; no ridge on the side of the rostrum; northern British Columbia specimens are rather uniform; those from southern part of the province are more variable, and differ less from Yukon skulls (Figs. 5*a*, 5*b*).

Great Plains adult skulls are those of females, and not significantly different from those of British Columbia females.

The following forms are recognizable in Canada.

GENUS *Erethizon* F. CUVIER (6, p. 432)

1822. Mém. Muséum hist. nat., Paris, 9 : 432. Type, *Hystrix dorsata* Linnaeus.

Erethizon dorsatum picinum Bangs

Labrador porcupine

1900. *Erethizon dorsatus picinus* Bangs. Proc. New England Zool. Club, 2 : 37. Type locality, L'Anse au Loup, Strait of Belle Isle, Labrador (3, p. 37).

1924. *Erethizon dorsatum picinum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Described as larger than *dorsatum*; tail a little shorter; colour plain black, without white-tipped hairs; skull rather larger; rostrum stouter, incisors broader and stronger, dull yellow instead of orange; molariform teeth smaller.

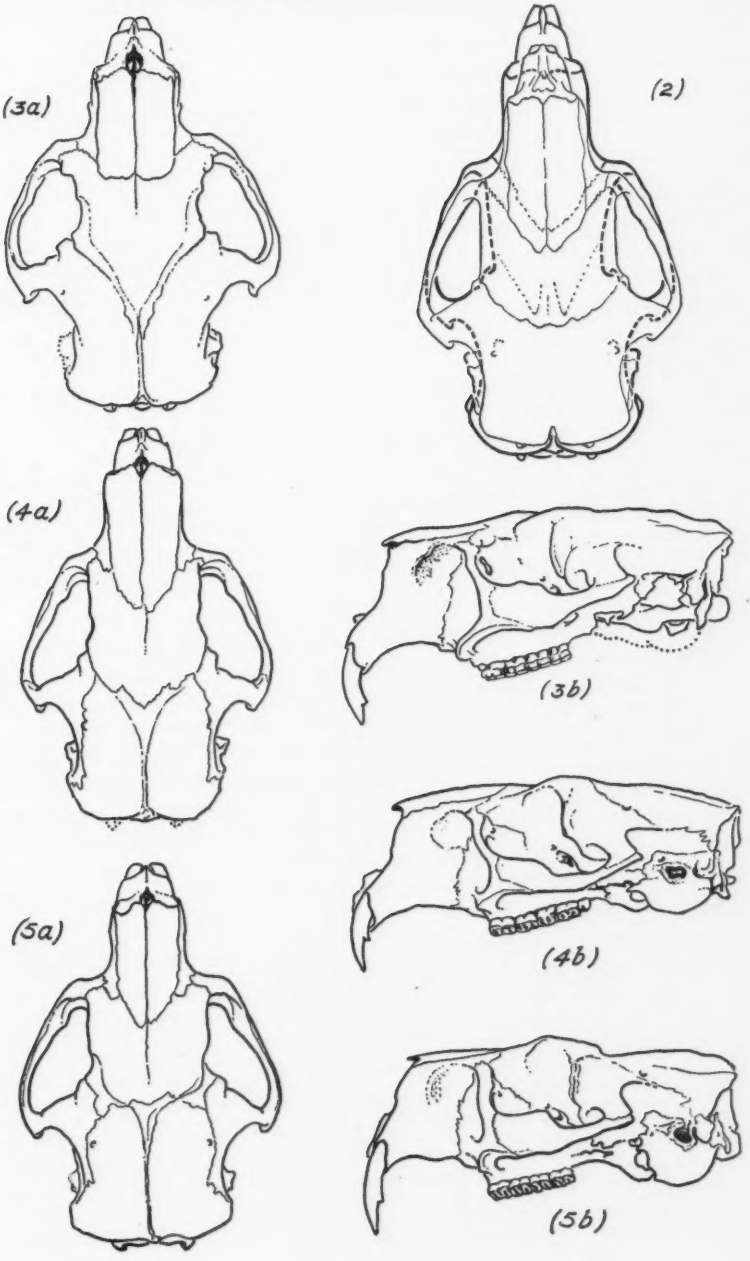
TABLE II

MEASUREMENTS OF *Erethizon dorsatum picinum* BANGS

Topotypes	External measurements			Skull measurements			
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Least inter-orbital breadth	Length of nasals
From Strait of Belle Isle, Labrador							
Five old males							
8835 *M.C.Z.* (Bangs collection)	725	172	115	—	—	—	—
8838 *M.C.Z.* "	753	195	97	—	—	—	—
8837 *M.C.Z.* "	757	192	106	—	—	—	—
8834 *M.C.Z.* "	757	204	97	—	—	—	—
8839 *M.C.Z.* "	790	166	124	99.6	76	34	35.6
Average	756.4	185.8	107.8	—	—	—	—
Two old females							
8840 *M.C.Z.*	697	183	104	—	—	—	—
8836 *M.C.Z.*	762	182	112	—	—	—	—
From Chimo, Que.							
N.M.C. No. 11058	—	—	—	90	68	31	30.5

Measurements of topotypes as given by Bangs (3, p. 38).

FIGS. 2 TO 4. *Skulls of Erethizon dorsatum.* FIG. 2. *E. dorsatum nigrescens, outlines of two skulls of adult males from Yahk, B.C. to show variation.* FIG. 3*a*. *E. dorsatum dorsatum, adult male, Gaspé, Que.* FIG. 3*b*. *E. dorsatum dorsatum, side view of 3a.* FIG. 4*a*. *E. dorsatum myops, young adult male, Teslin Lake, Yukon Territory.* FIG. 4*b*. *Side view of 4a.* FIG. 5*a*. *E. dorsatum nigrescens, adult male, Hot Springs, Dean Channel, B.C.* FIG. 5*b*. *Side view of 5a.*


(3a)
(2)
(4a)
(3b)
(5a)
(4b)
(5b)

Range

Common and generally distributed in Labrador and northeastern Quebec, from the St. Lawrence north to the semibarrens, west to Chimo but otherwise its western limits are unknown, though porcupines undoubtedly occur here and there over Ungava Peninsula south of the tundra.

Remarks

Lack of material prevents evaluation of this form. A single Chimo skull in the National Museum collection, compared with *dorsatum*, has the characters assigned to *picinum*. A single albino skin without skull, taken near Chimo, was received at the National Museum June 29, 1943, but gives no clue to the normal colour of pelage.

Specimens Examined

Two[1]. Quebec: Fort Chimo; two (one skin and one skull, from different animals).

Erethizon dorsatum dorsatum (Linnaeus)

Canada porcupine

1758. [*Hystrix*] *dorsata* Linnaeus. Systema naturae, ed. 10, 1 : 57 (10, p. 57). Type locality, Eastern Canada.

1822. *E*[*rethizon*] *dorsatum* F. Cuvier. Mém. Muséum hist. nat., Paris, 9 : 432 (6, p. 432).

1924. *Erethizon dorsatum dorsatum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Characterized by the small amount of yellowish or whitish tipping of the guard hairs, in most specimens confined to the nape, hip region, and tail; by little or no white on the head; by the pale yellowish-white colour of these tips; by the intense black or brownish black colour of the dark pigmented fur; by the slight swelling of the skull in the frontal region between the orbits, and the slight depression behind it; by the moderately developed cranial ridges running back in a straight line to fuse and form a short sagittal crest; by the nasals being short, subtruncate on their posterior end, or broadly rounded; by the presence of a ridge on the side of the rostrum on the premaxilla, running down diagonally from the base of the rostrum (Figs. 3*a*, 3*b*).

Range

Northeastern United States and Eastern Canada, from Nova Scotia and Gaspé to Manitoba and northern Saskatchewan; animals intergrading with *myops* in colour in the southern Yukon, and in skull characters in the Wood Buffalo Park, northern Alberta, according to our material.

There is considerable variation in the pelage of our 12 Nova Scotia, New Brunswick and Gaspé skins and nine Ontario and western Quebec skins. The specimens showing the least yellowish white have the ground colour black, or slightly brownish black, with small yellowish-white tips to a few of the guard hairs of the nape, the hips, and many of those fringing the tail. Eight

[1] *All specimens indicated as examined in the course of this study are in the mammal collection of the National Museum of Canada unless otherwise specified.*

TABLE III

MEASUREMENTS OF *Erethizon dorsatum dorsatum* (LINNAEUS)

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Length of nasals	Least interorbital breadth	Width of rostrum
Adult males								
Nova Scotia	800	210	110					
	830	230	100	101	72	31	33	23
Gaspé	788	181	108	103	—	33	31.5	26
Ontario	725	262	111	105	73.5	33	32	23
	737	230	108	98	—	30	32	24.5
Adult females								
Western Quebec	—	—	—	85	68	31	—	22.5
	—	—	—	—	—	34.5	—	23.5
Ontario	734	229	86	94	70	32	30	22.5
Manitoba, Thicket Portage	673	148	93	—	—	27.5	—	21.5
Adult, sex?								
New Brunswick	648	175	96	—	—	30	—	—
Western Quebec	—	—	—	91	—	31	33	23
				94	66	30.5	29.5	23
				99	—	33	29.5	20.5
				101	71	33.5	31	25
				89	—	28.5	31	—
				—	—	31.5	—	22
				99	—	35.5	34.25	23.5

of the Maritime specimens and six of the Ontario–western-Quebec skins differ slightly in having more yellowish-white–tipped guard hairs, with sometimes only a few scattered sparingly over the back. One Nova Scotia specimen has many of the guard hairs of the back narrowly tipped with yellowish white and with considerably more white showing in the spines of the head; two Ontario specimens have whitish-tipped guard hairs through most of the dorsal pelage, and one has them conspicuous over the whole back, flanks, and legs.

Two northwest Manitoba specimens differ somewhat in having the general tone slightly more brownish black; both have yellowish-white–tipped guard hairs scattered over the upper part of the body, in one of them most of the guard hairs are light-tipped. A skull from Sandilands Forest Reserve, southeast Manitoba, collected by J. Dewey Soper is definitely of this form, though it is that of a subadult female. Mr. Soper supplied us with a note that the species is common in Prince Albert National Park (north of Prince Albert), Sask., and that two specimens he saw there were typical *dorsatum*, being very dark, practically black.

There is variation in the skulls, but it is less than in skulls from southern British Columbia.

Specimens Examined

Nova Scotia: Shelburne Co., Barrington Passage, one; Guysborough Co., East Roman Valley, five.

New Brunswick: Gloucester Co. near Bathurst, three.

Quebec: Gaspé, one; Pontiac Co., 14.

Ontario: near Ottawa, one; Petawawa, one; Lanark Co., one; Lennox Co., one; Pancake Bay (Algoma District), one; Thunder Bay, one; York Factory, one (fragmentary skull only).

Manitoba: Thicket Portage (Hudson Bay Railway, mile 165), one; Herchmer, mile 412, one; Sandilands Forest Reserve, one[1].

Total, 34 specimens.

Erethizon dorsatum myops Merriam

Alaska porcupine

1900. *Erethizon epixanthus myops* Merriam. Proc. Wash. Acad. Sci. 2 : 27 (12, p. 27). Type locality, Portage Bay, Alaska Peninsula, Alaska.
1924. *Erethizon epixanthum myops* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Compared with *dorsatum* our specimens are characterized by the average browner colour of the dark pigmented fur; by the more abundant guard hairs; by the much greater amount of yellow in the guard hairs, by the distinctly rusty yellow tinge of this pale fur; by the narrower rostrum; by the longer nasals, more pointed posteriorly; by lack of swelling in the frontals; by a deeper depression in the frontal parietal area; by the cranial ridges being more developed over the orbit, moderately developed posteriorly; by moderate sagittal and occipital crests, and by the lack of a ridge on the side of the rostrum (Figs. 4*a*, 4*b*).

Range

Northern Alberta (Wood Buffalo Park) and southern Yukon to Alaska.

Remarks

We have no topotypical *myops* and refer the southern Yukon specimens tentatively to that form. Certainly they are different enough from our British Columbia series to represent another race; in the matter of outline of zygomatic arches, there is little more than a tendency toward the bowed conditions as described for *myops*.

A Wood Buffalo Park specimen is rather blackish generally, with abundant guard hairs, most of which are moderately tipped with rusty yellow; in appearance it is a black animal with yellow-tipped fur.

We have a series of 16 skins from southern Yukon, mostly from near Teslin Lake. Four of them have blackish or dark brownish general pigmentation with rather sparse guard hairs, two have only a few of the guard hairs on the nape and in the tail yellow-tipped; one has narrowly yellow-tipped guard

[1] *Kindly loaned by J. Dewey Soper of Winnipeg, Man.*

TABLE IV

MEASUREMENTS OF *Erethizon dorsatum myops* MERRIAM

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Length of nasals	Least inter-orbital breadth	Width of rostrum
Two adult males, Teslin Lake, Y.T. area				100	71.5	38	—	23
				96	—	40	—	21
Two adult females (?), Teslin Lake, Y.T.				104	70	40	28.5	21.5
				95	—	38	28	20
Adult female, Wood Buffalo Park, Alta.				93	73	31.5	28.5	21.5
Four adult males, Chitina River, Alaska	800	220	114	104	—	46.5	28.5	22
	750	170	97	99	70	36	29	22.25
	—	—	—	97	70	37	27	21.75
	—	—	—	107	71.5	38.75	31	24.5

hairs sparingly scattered over the back, and one has them fairly plentiful; they are very similar to eastern porcupines, differing only in the yellow averaging more rusty; on pelage alone one is indistinguishable from a Nova Scotia specimen even in the tint of the yellow.

Six other Yukon specimens are very different from the above; the dark pigmented fur is brownish and the extensive yellow tips of the profuse guard hairs are profusely distributed over the body, averaging rusty yellow in colour; the yellowish colour extends onto the hind part of the head. This gives the animal a distinctive brownish-yellow general appearance. In these six specimens four have the under surface of the tail mostly yellow, mixed with a little brownish; one has it mostly brownish; and one has it blackish. One Yukon specimen is blackish with abundant yellow-tipped guard hairs; the remaining five specimens are variably intermediate between the black and the yellow phases described above; the two Manitoba specimens would fit into this part of the series, except for having somewhat more whitish, rather than yellowish tips to the guard hairs.

Two Chitina glacier skins differ considerably from each other; one is pale brownish generally, with abundant yellow guard hairs, the yellowest porcupine of our collection; the other is like the intermediate Yukon specimens.

The above demonstrates that in the Teslin Lake area of Yukon Territory porcupines with eastern type and with western type pelage occur, and these are connected by intermediates. The western type of pelage occurs in a pronounced form as far eastward as Wood Buffalo Park.

In comparing the skulls, those from Teslin Lake (of which about eight are suitable for comparison) are all fairly distinct from eastern skulls on the characters given above.

However the skull of the Wood Buffalo Park specimen described above is especially illuminating in this connection. It is of the eastern porcupine type, in the short nasals, though they are rather pointed posteriorly, and in the swelling of the frontals; in the shape of the zygomatic arches it is of the *myops* type, the arches being more bowed. Though it is definitely *myops* in skin characters, in skull it shows a pronounced approach to *dorsatum*.

Specimens Examined

Alberta: Wood Buffalo Park, one.

Yukon Territory: vicinity of Teslin Lake, 15; Parent Creek, Duncan District, one; Whitehorse, one.

Alaska: Chitina River, four.

Total, 22 specimens.

Erethizon dorsatum nigrescens Allen

Dusky porcupine

1903. *Erethizon epixanthus nigrescens* Allen. Bull. Am. Museum Nat. Hist. 9 : 558. Type locality, Shesley River (45 miles from Telegraph Creek, B.C.) (2, p. 558).

1924. *Erethizon epixanthum nigrescens* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

The dark pigmented fur is blackish, as in *dorsatum*, from which it is distinguished by the more abundant guard hairs, with light tips averaging considerably more plentiful, scattered over the back; by the average orange-yellow rather than yellowish-whitish colour of these light tips; compared with our specimens of *myops* the general colour averages blackish, not brownish; the yellow tipping of the guard hairs is less extensive; and the colour of this tipping averages orange-yellow, rather than rusty yellow.

In the skulls, those from north British Columbia are not especially variable (Figs. 5*a*, 5*b*); those from south British Columbia are more so (Fig. 2). In some ways they represent a further divergence from the *dorsatum* skull than do *myops* skulls, from which latter they are distinguishable on the average by the greater development of the interior portion of the cranial ridge over the orbit giving a deeper frontal depression; by the moderately developed posterior portion of the cranial ridges curving together quickly, fusing together little back of the posterior margin of the frontals (instead of carrying far back, separately, and more nearly straight before meeting) giving a longer sagittal crest that is more developed; occipital crest higher; nasals long with more or less acute posterior end as in *myops*.

Range

British Columbia and Western Alberta, from Telegraph Creek and Jasper southward. Hall (8, p. 380), finds no difference between the skulls of British

TABLE V

MEASUREMENTS OF *Erethizon dorsatum nigrescens* ALLEN

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Basal length	Zygomatic breadth	Length of nasals	Interorbital breadth	Width of rostrum
Yahk, B.C., adult males	802	240	116	103	72.5	39	28	21.5
	833	237	124	101	75	42.5	36.5	22.5
	860	278	116	118	—	44	28.5	22.5
Chilcotin, B.C., adult male				112	76	37.5	32.5	24
Rossland, B.C., adult male	860	300	115	106.5	73	39	31	23
Bella Coola area, B.C., adult males	790	240	106	103.5	71.5	40	29.5	22
	850	200	110	100	72.5	36	31.5	21.5
	760	245	110	105	70.5	36	29.25	24.75
	850	270	111	100	77.5	36	32.5	24.5
				105	—	40	33	24.5
				106	75.5	38	31	24
Bella Coola area, B.C., adult females	740	210	101	95	68.5	35.5	28.5	20.5
	830	240	94	95	68	35.5	30	22
Okanagan, B.C., adult females	825	240	105	102	72.5	45.5	33.75	24.5
Waterton Park Lake, Alta., adult female				98	70	39	27.5	17

Columbia and of California taken porcupines, and separates the former as *nigrescens* on the basis of the orange-yellow colour of the bands on the long guard hairs. Our specimens in the average also have orange-yellow tips to the guard hairs.

Hall says also that in summer pelage the long hairs all over the back are broadly tipped with yellowish, while in winter the long hairs are light tipped mainly over the neck region and the sides of the tail, lacking over the midback and most of the rest of the upper parts.

However, our material shows this is individual variation, and the chief difference between winter and summer pelage is that in winter the under fur is long, concealing the spines, while in summer the spines are more or less exposed. The latter condition naturally exposes some of the white of the quills.

Remarks

Twelve of our 14 British Columbia skins agree in being generally blackish rather than brownish in ground colour and in having the yellow tipping of the long, abundant guard hairs more or less orange-yellow.

The amount of this yellow tipping varies considerably, from sparse narrow bands on some of the guard hairs to wide conspicuous bands on most of the guard hairs. The general appearance is of a black animal, with yellow-tipped guard hairs. Hall (8, p. 380) suggests that winter specimens are blacker. We have February and July specimens, and while the short under fur in the summer specimens allows more of the spines to be visible and more of the white of them to be seen (instead of being completely concealed) it does not change the appearance of the animal otherwise.

Five of the above specimens have the under surface of the tail black, with only a tinge of brownish at the base; one has it mostly yellowish, and the others are intermediate.

In respect to general blackness, and in the amount of yellow tipping to the guard hairs these 12 British Columbia specimens average about the same as the extreme eastern specimens (one from Ontario and one from Nova Scotia). However the yellow averages considerably deeper and more orange.

Two specimens, one from the Bella Coola area and one from Hagensborg are more brownish generally, with whitish extending onto the head, and with more yellow in the guard hairs giving the appearance of yellow-brown animals that are not unlike some of the intermediate Yukon specimens. Two skins from south British Columbia are less orange yellow, perhaps tending toward *epixanthum*; but one Yahk specimen has the yellow tips to the guard hairs light orange-yellow.

In regard to the skulls, the Bella Coola series are rather uniform—in their deep frontal depression; the projection over the orbit; high sagittal and occipital crests; and long sagittal crest; with the cranial ridges sharply pinched together. This is a further modification away from *dorsatum* of some of the characters seen in the south Yukon series (*myops*). However, those from elsewhere in British Columbia are more variable. Especially is this true of three skulls from near Yahk, in which the basal length, interorbital width, length and width of rostrum, the angle of projection of the incisors, and the height of the occipital crests vary greatly (Fig. 2). The characters of the skins from Rossland and Yahk however place them with this form. The Waterton Lake specimen is represented by a skull only, and is provisionally referred here. Davis (7, p. 344) refers specimens from southern Idaho to *epixanthum*, and Waterton Lakes Park specimens might be expected to be this form. According to Hall (8) the skulls of the two are indistinguishable.

Specimens Examined

Alberta: Jasper, one; Waterton Lakes Park, one (skull).

British Columbia: Yahk, three; Chilcotin, one; Rossland, one; Okanagan, one; Mackenzie Pass, Rainbow Mountains, one; Mount Brilliant, Rainbow Mountains, one; Dean Channel, six.

Total, 18 specimens.

Erethizon dorsatum epixanthum Brandt

California porcupine

1835. *Erethizon epixanthus* Brandt. Mém. acad. St-Pétersbourg (Sér. 6), 3 (Sci. nat. 1) : 390 (5, p. 390). Type locality, California (9, p. 27).

1924. *Erethizon epixanthum epixanthum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Great Plains specimens differ from any of our series of *myops* and *nigrescens* in the more greenish yellow, less orange or rusty yellow of the light tipping of the guard hairs. The available adult skulls are of females and, from those of our specimens of *nigrescens*, differ chiefly in the cranial ridges being straight and meeting farther back on the skull; however females generally show less differences than do males, and we have fewer skulls of females to show the range of variation.

The variations found in both external characters and skull characters in the Great Plains region may be due to the fact that this large area has little country suitable for porcupines, and small inbred groups in this area of intergradation may be indicated.

Two of the adult skins from western escarpment of Cypress Hills (Lodge Creek, Alta.) are very similar to some of the Yukon specimens referred to *myops* but the general tone of the yellow of guard hairs is greenish yellow rather than rusty yellow; one of these specimens has the under surface of the tail mostly yellow, mixed with a little brown, and in the other the tail is mostly brownish black. A smaller subadult specimen from the same locality is blackish brown with conspicuous whitish tips to the guard hairs on nape and rump and a few on the back, similar to the Manitoba specimens, but all the Lodge Creek specimens have the typical long, narrow nasals of the western porcupines.

Two adult female skins from Lonesome Butte, Sask., about 200 miles farther east on the Great Plains, compared with Lodge Creek specimens, are much paler in general appearance, with heavier growth of pale greenish yellow guard hairs from head to tail and with face and throat noticeably lighter

TABLE VI

MEASUREMENTS OF *Erethizon epixanthum epixanthum* BRANDT

	External measurements			Skull measurements				
	Total length	Tail	Hind foot	Condylo-basal length	Zygo-matic breadth	Nasal length	Inter-orbital width	Width of rostrum
Lodge Creek, Alta., adult females	790	220	120	98	71.5	43	34	26
	765	205	100	96	69	37.5	39	22

gray in colour; the skulls also approach the eastern porcupine in comparatively greater breadth and shortness; nasals shorter, one specimen (No. 8320) having broad, short nasals only 30 mm. long.

Range

From western United States into at least the Cypress Hills area of southeastern Alberta and southwestern Saskatchewan, Canada.

Remarks

Swenk (17, pp. 115–125) described *bruneri* from Nebraska, the type from Mitchell, Scottsbluff County, Neb., and paratype from near Mullen, Cherry County, Neb.; he referred one specimen from Kansas, three from Wyoming, one from eastern Montana to the same form on the basis of recorded skull measurements. Amongst other characters it is supposed to be larger than either *epixanthum* or *nigrescens*. Our specimens compared with British Columbia material show no appreciable difference in size, and on the basis of shade of yellow are referred to *epixanthum*, though we have no topotypical *epixanthum* nor *bruneri* for comparison. Davis (7, p. 344) records specimens from southern Idaho as *epixanthum*.

"Yellow-haired" porcupines have been recorded taken as far east as the Turtle Hills, N.D., and might be expected in southern Manitoba where porcupines are known to occur; specimens from that area would be especially interesting.

Specimens Examined

Alberta: Lodge Creek, near western edge of Cypress Hills, three.

Saskatchewan: Lonesome Butte, southwest of Wood Mountain, near Montana boundary, two.

Total, five specimens.

References

1. ALLEN, J. A. Monographs of North American Rodentia. Rept. U.S. Geol. Survey Terr. 11 : 379-398. 1877.
2. ALLEN, J. A. Mammals collected in Alaska and northern British Columbia by the Andrew J. Stone expedition of 1902. Bull. Am. Museum Nat. Hist. 19 : 521-567. 1903.
3. BANGS, O. Three new rodents from southern Labrador. Proc. New England Zool. Club, 2 : 35-41. 1900.
4. BANGS, O. List of the mammals of Labrador. *In* Grenfell, W.T. *et al.*, Labrador, the country and the people. The Macmillan Company, New York. 1900.
5. BRANDT, J. F. v. Mammalium rodentium exoticorum novarum vel minus rite cognitorum. Mus. Acad. Zool. descriptiones et icones. (1834). Mém. acad. St-Pétersbourg (Sér. 6), 3 (Sci. nat. 1) : 357-442. 1835.
6. CUVIER, F. Mém. Muséum hist. nat., Paris, 9 : 432. 1822.
7. DAVIS, W. B. The recent mammals of Idaho. The Caxton Printers, Caldwell, Idaho. 1939.
8. HALL, E. R. Mammals collected by T. T. and E. B. McCabe in the Bowron Lake region of British Columbia. Univ. Calif. Pub. Zool. 40 : 363-386. 1934.
9. HOLLISTER, N. Mammals of the Alpine Club expedition to the Mount Robson region. Can. Alpine J., Spec. No., 1-44. 1912.

10. LINNAEUS, C. v. Caroli Linnaei Systema Naturae per Regna tria naturae, Secundum Classes, Ordines, Genera, Species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio Decima, Reformata. Holmiae, Impensis Direct, Laurentii Salvii. 1758.

11. MEARNS, E. A. Descriptions of six new mammals from North America. Proc. U.S. Natl. Museum, 19 : 719-724. 1897.

12. MERRIAM, C. H. Descriptions of twenty-six new mammals from Alaska and British North America, 13-20. Papers from the Harriman Alaska expedition. Proc. Wash. Acad. Sci. 2 : 1-694. 1900.

13. MILLER, G. S., JR. List of North American recent mammals, 1923. U.S. Natl. Museum, Bull. 128. 1924.

14. SCHULTZ, A. H. Bregmatic fontanelle bones in mammals. J. Mammal. 4 : 65-77. 1923.

15. SWARTH, H. S. Birds and mammals of the Skeena River region of northern British Columbia. Univ. Calif. Pub. Zool. 24 : 315-394. 1924.

16. SWARTH, H. S. Mammals of the Atlin region, northwestern British Columbia. J. Mammal. 17 : 398-405. 1936.

17. SWENK, M. H. On a new subspecies of porcupine from Nebraska. Univ. Nebraska Studies, 16 : 115-125. 1916.

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